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The Effect of Dietary Protein and Energy Level Upon the Nitrogen Components in the Urine of the Domestic Hen.

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THE EFFECT OF DIETARY PROTEIN AND ENERGY LEVEL UPON THE NITROGEN COMPONENTS IN THE URINE OF THE DOMESTIC HEN

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ABSTRACT

SURGICAL MODIFICATIONS WERE MADE WHICH PERMITTED THE SEPARATE COLLECTION OF FECES AND URINE FROM CHICKENS. THE TECHNIQUES WERE REFINED AND UTILIZED IN A FUNDAMENTAL STUDY OF NITROGEN METABOLISM.

IT WAS FOUND NECESSARY TO ADD BORIC ACID AS A PRESERVATIVE TO THE URINE AND FECES TO OBTAIN QUANTITATIVE RECOVERY OF NITROGEN.

THERE WERE NO DIFFERENCES IN THE RESPONSE TO TREATMENT AMONG NORMAL HENS, HENS WITH EXTERIORIZED RECTA, AND HENS WITH EXTERIORIZED URETERS. HENS WITH EXTERIORIZED RECTA WERE THE BIRDS OF CHOICE FOR NUTRITIONAL EXPERIMENTS BECAUSE EXCRETA FROM THESE HENS WERE EASIER TO COLLECT.

IN AN EXPERIMENT WITH ISO-PROTEIN RATIONS IT WAS FOUND THAT AS THE RATION ENERGY LEVEL DECREASED FROM 900 TO 800 CALORIES PER POUND OF PRODUCTIVE ENERGY, THE AMOUNT OF URINARY NITROGEN INCREASED, THE PERCENT OF NITROGEN DIGESTED DECREASED, AND THE FEED INTAKE DECREASED. THERE WAS A CHANGE IN THE URINARY NITROGEN EXCRETION; THE URIC ACID NITROGEN EXCRETION INCREASED, THE AMMONIA NITROGEN DECREASED, THE UREA NITROGEN INCREASED, THE CREATINE NITROGEN REMAINED CONSTANT, AND THE AMINO ACID NITROGEN WAS DECREASED SLIGHTLY.

A STUDY WAS MADE IN WHICH THE HENS WERE ALLOWED TO EAT A LIMITED AMOUNT OF NO-PROTEIN DIET CALCULATED TO FURNISH THE DAILY ENERGY REQUIREMENT AND SUPPLEMENTED WITH DAILY DECREASING INCREMENTS OF EXTRACTED WHOLE EGG (13.7 TO ZERO PERCENT). IN THIS EXPERIMENT THE ABSORBED NITROGEN SHOWED A LINEAR DECREASE AS THE EGG PROTEIN WAS REDUCED. THE URIC ACID

AND AMMONIA NITROGEN DECREASED THROUGHOUT THE EXPERIMENT WITH A CHANGE IN THE DAILY RATE OF DECREASE AFTER THE SEVENTH DAY (5.3 PERCENT SUPPLEMENTARY EGG PROTEIN). THE UREA NITROGEN EXCRETION DECREASED UNTIL THE SEVENTH DAY, PEAKED THE EIGHTH DAY, AND DECREASED FROM THEN TO THE LAST DAY OF THE EXPERIMENT.

THE CREATINE EXCRETION DROPPED THE FIRST DAY OF DEPLETION, INCREASED TO THE STARTING VALUE THE EIGHTH DAY, AND DECREASED ON THE FIRST DAY OF NO-PROTEIN FEEDING. THROUGHOUT THE EXPERIMENT THE CREATINE EXCRETION WAS ERRATIC AND INDICATED THAT THE PROTEIN METABOLISM WAS CHANGING.

THE AMINO ACID NITROGEN AND UNKNOWN COMPONENT (3) TENDED TO REMAIN CONSTANT.

THE URINARY CARBOHYDRATE EXCRETION, AS MEASURED BY THE ANTHRONE METHOD, INCREASED AS THE PROTEIN LEVEL DECREASED. THE PEAK VALUE, ON THE FIRST DAY OF NO-PROTEIN FEEDING, (TWELFTH DAY OF THE EXPERIMENT) WAS 3400 MGMS. AS COMPARED TO 250 MGMS. ON THE FIRST DAY OF THE STUDY.

A STUDY OF THE DATA OF BOTH EXPERIMENTS LEADS TO THE ADVANCEMENT OF TWO HYPOTHESES OF NITROGEN METABOLISM, NAMELY:

(1) IT IS NOT NECESSARILY THE AMOUNT OF PROTEIN IN A RATION BUT THE RELATIONSHIP OF THE PROTEIN TO OTHER NUTRIENTS WHICH DETERMINES THE CHARACTERISTICS OF THE URINARY NITROGEN EXCRETION.

(2) IN ORDER TO CALL A URINE "NORMAL" ONE MUST RIGIDLY DEFINE A "NORMAL" DIET. IN THESE RESPECTS, NO EVIDENCE WAS FOUND WHICH WOULD REFUTE THE CONCLUSIONS OF FOLIN, NEITHER HIS LAWS FOR THE COMPOSITION OF URINE NOR HIS THEORY OF ENDOGENOUS AND EXOGENOUS NITROGEN METABOLISM.

INTRODUCTION

For years scientists have been working to improve the proteins consumed by man and animals.

Recent research at the Louisiana State University has produced a valuable research tool for those scientists working with avian species, namely, a surgical procedure for the separate collection of feces and urine. Such surgical procedures have been used before but success was limited and no proof was offered that the surgically modified bird retained the same metabolic and physiological characteristics as was exhibited before surgery. The surgical procedures were determined and were in use, but it was necessary in the work presently presented to show that surgically modified hens responded to ration treatment in the same manner as their normal sisters and to refine the techniques for the separate, quantitative collection of urine and feces.

Of the three experiments presented only two were of particular value other than the defining of collection techniques. The first of these two experiments was to determine the effect of the energy level of the ration on the nitrogen components of the urine and the second experiment was to determine the effect of the protein level in the ration on these same nitrogen components.

REVIEW OF LITERATURE

VERY LITTLE WORK HAS BEEN DONE ON THE NITROGEN COMPOSITION OF URINES WHICH INVOLVES ALL OF THE COMPONENTS AT ONE TIME. THE EARLY CLASSICAL WORK IN THE FIELD WAS DONE BY DR. OTTO FOLIN (1905A). HE ANALYZED THIRTY "NORMAL" HUMAN URINES FOR AMMONIA, UREA, URIC ACID, AND CREATININE NITROGEN. WITH A STANDARD DIET COMPOSED OF 119 GM. PROTEIN, 148 GM. FAT, AND 225 GM. CARBOHYDRATE, HE FOUND THAT WITHIN A CLOSE DEVIATION, THE INDIVIDUAL COMPONENTS OF THE URINE REPRESENTED THE SAME PERCENT OF URINARY NITROGEN EACH DAY FOR FIVE DAYS. THE VARIATION FROM PERSON TO PERSON WAS SURPRISINGLY SMALL.

CONCERNING THE DIET HE USED, FOLIN HAD THIS TO SAY:

"IN REGARD TO THE PROTEIN, IT CORRESPONDS ALMOST EXACTLY WITH THE VALUE DEMANDED BY VOIT FOR A MAN WEIGHING 70 KGM.; AND SINCE FOUR OUT OF THE SIX NORMAL PERSONS EXPERIMENTED UPON WEIGHED LESS THAN 70 KGM. AND THE OTHER TWO WEIGHED NO MORE THAN 70 KGM., THE DIET MAY BE SAID TO BE LIBERAL RATHER THAN TOO SCANTY WITH RESPECT TO ITS PROTEIN CONTENT.----"

----"IT IS NOT MY INTENTION TO DEVOTE ANY SPACE TO DISCUSSION OF THESE THIRTY NORMAL URINES, FOR THE VERY REASON THEY CORRESPOND SO CLOSELY TO WHAT WE HAVE BEEN ACCUSTOMED TO CONSIDER NORMAL."

FOLIN (1905B) EXTENDED HIS STUDY OF URINE STILL FURTHER BY DETERMINING THE EFFECT OF "NON-STANDARD" DIETS. HIS STATEMENTS ON THIS ARE AS FOLLOWS:

"THIS PAPER IS DEVOTED TO A STUDY OF HUMAN URINES OBTAINED FROM DIETS WHICH ARE AS DIFFERENT FROM THE SO-CALLED STANDARDS OF DIET AS THEY COULD BE MADE, AND THE RESULTS ARE BELIEVED TO FURNISH A MORE OR LESS NEW POINT OF VIEW FROM WHICH TO EXAMINE THOSE STANDARDS.----"

THE URINE OF DR. E. VAN SOMEREN WAS OF PARTICULAR INTEREST. DR. VAN SOMEREN ROUTINELY ATE A NON-STANDARD MIXED DIET CONSISTING LARGELY OF VEGETABLES, BREAD, CRACKERS, CREAM, AND CANDIES. DR. VAN SOMEREN WAS INDUCED TO CHANGE HIS DIET TO THE NITROGEN RICH DIET WHICH WAS USED IN THE PREVIOUS EXPERIMENT (FOLIN 1905A). AFTER TWO DAYS HIS URINE (SHOWN IN THE RIGHT COLUMN OF THE TABLE BELOW) BEGAN RESEMBLING THE "NORMAL" VALUES EVEN THOUGH HE COULD ONLY CONSUME ABOUT 50 PERCENT OF THE USUAL AMOUNT OF THE DIET.

"THE WIDE VARIATIONS IN THE COMPOSITION OF DR. VAN SOMEREN'S URINES ARE CHIEFLY DUE TO THE TEMPORARY CHANGE IN DIET ON JANUARY 30 AND 31 MENTIONED ABOVE."

DURING THESE TWO DAYS HE LOST WEIGHT BUT UPON RETURNING TO HIS OLD DIET HE BEGAN TO GAIN.

	NORMAL	DR. E. V.S.
TOTAL N.	14.8 - 18.2 GM.	4.8 - 8.0 GM.
UREA N.	66.3 - 89.4 %	62.0 - 80.4 %
AMMONIA N.	3.3 - 5.1 %	4.2 - 11.7 %
KREATININ N.	3.2 - 4.5 %	5.5 - 11.1 %
URIC ACID N.	0.5 - 1.0 %	1.2 - 2.4 %
UNDETERMINED N.	2.7 - 5.3 %	4.8 - 14.6 %

----"IT MAY, THEREFORE BE POSITIVELY STATED AS A PRINCIPLE IN THE CHEMISTRY OF METABOLISM THAT THE DISTRIBUTION OF THE NITROGEN IN URINE AMONG UREA AND THE OTHER NITROGENOUS CONSTITUENTS DEPENDS ON THE ABSOLUTE AMOUNT OF TOTAL NITROGEN PRESENT."

IN THIS SAME PAPER FOLIN REPORTED A FEEDING TRIAL WITH HUMANS IN WHICH HE FED A LOW PROTEIN, STARCH AND CREAM DIET FOR SEVEN DAYS WITH A TWO DAY RETURN TO THE PROTEIN RICH DIET. THE AMOUNT OF NITROGEN IN THE URINE DECREASED DAILY. THE UREA NITROGEN AS A PERCENTAGE OF URINARY NITROGEN DECREASED DAILY AND THE PERCENTAGE OF AMMONIA, URIC ACID, AND CREATININE NITROGEN INCREASED DAILY WHILE THE SUBJECTS WERE ON THE LOW PROTEIN DIET. FOLIN MADE THE FOLLOWING COMMENTS ON THE LATTER THREE NITROGEN COMPONENTS OF URINE:

----"THE ABSOLUTE QUANTITY OF KREATININ ELIMINATED IN THE URINE ON A MEAT-FREE DIET IS A CONSTANT QUANTITY DIFFERENT FOR DIFFERENT INDIVIDUALS, BUT WHOLLY INDEPENDENT OF QUANTITATIVE CHANGES IN THE TOTAL AMOUNT OF NITROGEN ELIMINATED."

----"WHEN THE TOTAL AMOUNT OF PROTEIN-METABOLISM IS GREATLY REDUCED, THE ABSOLUTE QUANTITY OF URIC ACID IS DIMINISHED, BUT NOT NEARLY IN PROPORTION TO THE DIMINUTION IN THE TOTAL NITROGEN, AND THE PERCENT OF THE URIC ACID NITROGEN IN TERMS OF THE TOTAL NITROGEN IS THEREFORE MUCH INCREASED."

"WITH PRONOUNCED DIMINUTION IN THE PROTEIN METABOLISM (AS SHOWN BY THE TOTAL NITROGEN IN THE URINE), THERE IS USUALLY, BUT NOT ALWAYS, AND THEREFORE NOT NECESSARILY, A DECREASE IN THE ABSOLUTE QUANTITY OF AMMONIA ELIMINATED. A PRONOUNCED REDUCTION OF THE TOTAL NITROGEN IS, HOWEVER, ALWAYS ACCOMPANIED

BY A RELATIVE INCREASE IN THE AMMONIA NITROGEN, PROVIDED THE FOOD IS NOT SUCH AS TO YIELD AN ALKALINE ASH."

FOLIN TREATS THE UNDETERMINED NITROGEN IN THE FOLLOWING MANNER:

----"I.E., THE ABSOLUTE QUANTITY OF UNDETERMINED NITROGEN DECREASES UNDER THE INFLUENCE OF THE STARCH AND CREAM DIET, BUT IN PERCENT OF THE TOTAL NITROGEN THERE IS ALWAYS AN INCREASE."

FOLIN (1905c) UTILIZED THESE OBSERVATIONS TO FORM "A THEORY OF PROTEIN METABOLISM".

"IT IS CLEAR THAT THE LAWS GOVERNING THE COMPOSITION OF URINE REPRESENTED ONLY THE EFFECTS OF OTHER MORE FUNDAMENTAL LAWS GOVERNING THE KATABOLISM OF PROTEIN IN THE ANIMAL ORGANISM. FROM THE VARIATIONS IN THE PERCENTAGE COMPOSITION OF URINE DESCRIBED BY THE ABOVE GENERALIZATIONS, IT WOULD SEEM THEREFORE THAT SOME CONCLUSION MIGHT BE DRAWN IN REGARD TO THE NATURE OF PROTEIN METABOLISM.----"

-----"IT IS CLEAR THAT THE METABOLIC PROCESSES RESULTING IN THE PRODUCTS WHICH TEND TO BE CONSTANT IN QUANTITY APPEAR TO BE INDISPENSABLE FOR THE CONTINUATION OF LIFE; OR, TO BE MORE DEFINITE, THOSE METABOLIC PROCESSES PROBABLY CONSTITUTE AN ESSENTIAL PART OF THE ACTIVITY WHICH DISTINGUISHES LIVING CELLS FROM DEAD ONES. I WOULD THEREFORE CALL THE PROTEIN METABOLISM WHICH TENDS TO BE CONSTANT, TISSUE METABOLISM OR ENDOGENOUS METABOLISM, AND THE OTHER, THE VARIABLE PROTEIN METABOLISM, I WOULD CALL THE EXOGENOUS OR INTERMEDIATE METABOLISM."

-----"THE ENDOGENOUS METABOLISM SETS A LIMIT TO THE LOWEST

LEVEL OF NITROGEN EQUILIBRIUM ATTAINABLE."

FOLIN ALSO ADVANCED THE THEORY THAT PROTEIN CAN BE STORED AND THAT PLASMA NITROGEN IS A STORAGE POINT FOR NITROGEN.

MITCHELL (1924) BASED HIS REFINEMENT OF THE THOMAS TECHNIQUE FOR DETERMINING THE BIOLOGICAL VALUE OF PROTEIN UPON THE THEORY OF FOLIN. HIS BASE-LINE URINARY NITROGEN VALUE WAS THE ENDOGENOUS NITROGEN EXCRETION DEFINED BY FOLIN. HE ALSO DISCOVERED THAT:

> "THE "METABOLIC NITROGEN" OF THE FECES ON A PROTEIN-CONTAINING DIET IS RELATED TO THE AMOUNT OF FOOD CONSUMED, AND MAY BE MEASURED BY THE TOTAL EXCRETION OF FECAL NITROGEN ON A NITROGEN-FREE DIET. THE LATTER MAY BE USED WITH THE MOST CONFIDENCE WHEN THE ROUGHAGE CONTENT OF THE NITROGEN-FREE DIET APPROXIMATES THAT OF THE PROTEIN-CONTAINING DIET."

MITCHELL FOUND THAT ENDOGENOUS URINARY NITROGEN EXCRETION REMAINED VERY STABLE AFTER RATS HAD BEEN ON A NITROGEN-FREE DIET FOR ABOUT A WEEK.

WANG ET AL. (1930) STUDIED THE INFLUENCE OF THE PROTEIN LEVEL ON THE BASAL METABOLISM AND BLOOD AND URINE CHEMISTRY ON SIX NORMAL WOMEN VARYING IN AGE FROM 17 TO 36 YEARS. THE SUBJECTS RECEIVED FOR FIVE WEEKS A HIGH PROTEIN DIET CONTAINING TWO GM. PROTEIN, AND SUFFICIENT CARBOHYDRATE AND FAT TO MAKE 40 CALORIES, PER KILO. OF BODY WEIGHT. THE PROTEIN LEVEL WAS REDUCED WITH CALORIC LEVEL HELD AT 40 FOR A PERIOD OF THREE WEEKS AT WHICH TIME THE PROTEIN INTAKE WAS 0.6 GM. PER DAY. THE SUBJECTS WERE ALLOWED TO CHOOSE THEIR OWN FOOD FOR TWO WEEKS. NO MARKED DIFFERENCE WAS FOUND IN THE BASAL METABOLIC RATE OF THE SUBJECTS DURING THE THREE PERIODS.

"As was expected, the total urinary nitrogen, urea nitrogen, ammonia nitrogen, uric acid and creatine varied directly with the protein intake. Creatinine remained constant throughout the investigation."

Smuts (1935) studied the possible relationship of basal metabolic rate and endogenous nitrogen excretion. Working with guinea pigs, mice, rabbits and pigs, he found a relationship between B M R, creatinine excretion, and protein requirement for maintenance. According to Smuts the protein requirement for maintenance may be estimated as follows:

$P = .88M^{.734}$

P = Absolute protein requirement for maintenance in grams.

M = Body mass in kilograms.

Murlin et al. (1948) correlated biological values of proteins and the percent creatinine nitrogen in the urine of mature males. There was a high correlation in all instances ($R = 0.972$). The various test proteins were fed at a low enough level to produce a negative nitrogen balance.

Schoenheimer et al. (1939) attacked the Folin theory of protein metabolism. They used amino acids labeled with the heavy isotope of nitrogen and found that some 57 percent of the labeled nitrogen of leucine remained in rat protein after a three day feeding trial. Only 27.6 percent was excreted in the urine during the period. The isotope appeared in protein amino acids other than leucine. Lysine contained very little of the labeled nitrogen. This work showed clearly that the nitrogen within the body was in a dynamic state and proteins were being constantly

BUILT AND DEGRADED.

CONCERNING ENDOGENOUS AND EXOGENOUS METABOLISM THEY WROTE:

"IT IS SCARCELY POSSIBLE TO RECONCILE OUR FINDINGS WITH ANY THEORY WHICH REQUIRES A DISTINCTION BETWEEN THESE TWO TYPES OF NITROGEN.----"

BURROUGHS ET AL. (1940) REEMPHASIZED THE CONSTANCY OF THE ENDOGENOUS NITROGEN EXCRETION AS THEORIZED BY FOLIN. ADDED AMINO ACID MIXTURES AND EGG PROTEIN, AT LOW LEVELS, DID NOT VARY URINARY NITROGEN EXCRETION AS COMPARED TO TWO PERIODS OF FEEDING A NITROGEN-FREE DIET (BEFORE AND AFTER).

MITCHELL (1955) PROVIDED MORE EXPERIMENTAL EVIDENCE TO SUPPORT THE FOLIN THEORY. A VERY GOOD SUMMATION OF HIS WORK AND CONCLUSIONS APPEAR IN THE FOLLOWING QUOTE:

"EVIDENCE HAS BEEN PRESENTED FROM EXPERIMENTS ON GROWING RATS AND GROWING PIGS RECEIVING DIFFERENT DIETARY PROTEINS OF HIGH NUTRITIONAL VALUE AT DIFFERENT LEVELS OF INTAKE TO THE EFFECT THAT (1) THE URINARY NITROGEN OUTPUT OF A NORMAL ANIMAL ON A PRACTICALLY NITROGEN-FREE DIET IS NOT NECESSARILY DEPRESSED BY THE INGESTION OF DIETARY PROTEIN OR DIETARY METHIONINE. UNDER CERTAIN CONDITIONS, APPARENTLY ASSOCIATED WITH AN ACCELERATED ENDOGENOUS NITROGEN METABOLISM, SUCH A DEPRESSION HAS BEEN REPORTED. IT ALSO SHOWS THAT (2) A CONSTANT FRACTION OF THE ABSORBED NITROGEN ABOVE THE NITROGEN OUTPUT ON A NITROGEN-FREE DIET IS RETAINED IN THE BODY OF THE GROWING ANIMAL, IMPLYING THAT THE ENDOGENOUS OUTPUT OF URINARY NITROGEN CONTINUES AT A CONSTANT LEVEL THROUGHOUT SUBSEQUENT PERIODS OF INCREASING PROTEIN INTAKE.

THE EXPERIMENTAL EVIDENCE PRESENTED, OR CITED, IN THIS PAPER, ESTABLISHES CLEARLY THE VALIDITY OF FOLIN'S CONCEPT OF DICHOTOMY IN PROTEIN METABOLISM INTO ENDOGENOUS AND EXOGENOUS TYPES. NO WELL DEMONSTRATED FINDINGS IN THE AREA OF PROTEIN METABOLISM HAVE BEEN FOUND CONTRADICTORY TO THIS CONCEPT; ON THE CONTRARY MANY ARE BEST EXPLAINED ON THIS BASIS. RECENT RESEARCH WITH ISOTOPE TRACERS HAS CLARIFIED THE NATURE OF THESE DICHOTOMIC ENTITIES WITHOUT DESTROYING THEIR IDENTITIES."

THE OBSERVED FACTS HAVE EMPHASIZED THE IMPORTANCE OF CREATININE AS A BIOLOGICAL CONSTANT, AND AS SUCH MIGHT BE USEFUL IN OTHER STUDIES ON PROTEIN METABOLISM.

MURLIN ET AL. (1953) STUDIED THE CORRELATION BETWEEN BIOLOGICAL VALUE OF PROTEIN AND THE PERCENT OF CREATININE NITROGEN IN THE URINE OF TWO DOGS. THE CORRELATION BETWEEN BIOLOGICAL VALUE AND THE PERCENT CREATININE NITROGEN WAS HIGH (0.988 AND 0.946).

ALLISON AND LEONARD (1941) DETERMINED THE EFFECTS OF ESTROGEN AND THYROIDECTOMY ON THE EXCRETION OF CREATINE AND CREATININE IN FEMALE RATS. THEY COMPARED URINE COMPOSITION OF NORMAL, CASTRATE, AND THYROIDECTOMIZED RATS.

WITHIN EACH GROUP, CREATININE EXCRETION REMAINED CONSTANT. THERE WAS NO APPARENT EFFECT OF THE ESTROUS CYCLE ON THE EXCRETION OF CREATININE. COMPARED TO NORMAL RATS, CASTRATION LOWERED THE CREATININE EXCRETION SLIGHTLY AND THYROIDECTOMY LOWERED THE EXCRETION MARKEDLY. THE ADMINISTRATION OF ESTROGEN HAD NO EFFECT ON THE CREATININE EXCRETION OF THE THYROIDECTOMIZED RATS, HOWEVER, IT INCREASED THE CREATININE EXCRETION OF THE CASTRATE ANIMALS.

Because of the anatomy of the fowl, only limited studies have been conducted on the urinary nitrogen components of the avian species. Sharpe (1912) cannulated the ureters of anesthetized hens and made certain diuretic studies. These hens were maintained on a diet of oats and water. His conclusions were:

> "The urine of hens is usually abundant and clear as it leaves the ureters, and its water content must be largely reabsorbed from the bowel. The ordinary diuretics produce increases in urine flow similar to that found in other animals."

Sharpe determined that 30 percent of the urinary nitrogen was uric acid nitrogen and 5.6 percent was ammonia nitrogen.

Davis (1927) collected urine from anesthetized hens by means of a catheter placed in the urodeum near the openings of the ureters. The average partition of the nitrogen components appear in the following table.

Nitrogen constituents in 100 cc of hen urine

Total N, mg.	Uric Acid N. mg.	Urea N. mg.	Ammonia N. mg.	Creatine-Creatinine N. mg.	Undetermined N. mg.
100	62.9	10.4	17.3	8.0	1.4

Davis also determined that anesthesia increased urine flow.

Coulson and Hughes (1930) used the method of Davis to collect urine from hens in an attempt to arrive at a constant which would be useful in the determination of the biological value of proteins for chickens. They determined urea by the method of Folin and Youngberg, ammonia by the Folin and Bell Permutite method, uric acid by the Folin and Wu silver lactate method and allantion by the Harding and Young method. According to this study the percentage composition of the urinary nitrogen was uric acid

NITROGEN, 65.80 PERCENT, PURINE (OTHER THAN URIC ACID) NITROGEN, 9.57 PERCENT, UREA NITROGEN, 6.45 PERCENT, AMMONIA NITROGEN, 7.58 PERCENT, CREATINE AND CREATININE NITROGEN, 4.56 PERCENT, AND POSSIBLE ALLANTION NITROGEN, 3.83 PERCENT. THE ALLANTION NITROGEN WAS LISTED A "POSSIBLE" BECAUSE THE METHOD WAS NOT SPECIFIC.

ST. JOHN ET AL. (1932) DETERMINED THE BIOLOGICAL VALUE OF SOME PROTEINS IN NORMAL CHICKENS BY USING A CHEMICAL METHOD OF SEPARATING THE AMMONIA AND URIC ACID NITROGEN FROM THE OTHER NITROGEN IN THE DROPPINGS. THEY MULTIPLIED THE NITROGEN IN THESE COMPONENTS BY THE FACTOR 1.25, BASED ON THE PAPER OF COULSON AND HUGHES. BECAUSE OF THE DIFFICULTY IN CHEMICALLY SEPARATING URINE AND FECES AND BECAUSE OF THE TEDIOUS CHEMICAL PROCEDURES, THIS METHOD WAS NOT WIDELY USED.

IN THE PAST, SURGICAL PROCEDURES TO ESTABLISH SEPARATE EXTERIOR OPENINGS FOR THE GASTROINTESTINAL AND THE URINARY TRACTS WERE NOT POPULAR BECAUSE IT WAS DIFFICULT TO MAINTAIN SUCH BIRDS IN A GOOD STATE OF HEALTH. THIS WAS BELIEVED TO BE DUE TO THE FACT THAT BIRDS ABSORBED URINARY WATER IN THE CLOACA AND THE RECTUM. THE EVIDENCE FROM THE CATHETERIZATION EXPERIMENTS HAD INDICATED THAT HENS EXCRETED UP TO 1000 MLS. OF URINE PER 24 HOURS AND THAT A GREAT DEAL OF THE WATER WAS REABSORBED.

HESTER ET AL. (1940) USED VARIOUS CATHETERIZATION TECHNIQUES AND EXTERIORIZED THE URETERS OF SOME HENS. THEY FOUND THAT URINE EXCRETION FROM THE SURGICALLY MODIFIED HENS SELDOM EXCEEDED 125 ML. THIS CAST DOUBT ON THE THEORY THAT WATER WAS ABSORBED IN THE CLOACA.

HART AND ESSEX (1942) PERFORMED TWO TYPES OF SURGERY, THE EXTERIORIZATION OF THE URETERS AND ARTIFICIAL ANUS. THEY WORKED WITH URINE EXCRETION AND WATER BALANCE. THEY ALSO MADE A FEW URIC ACID DETERMINATIONS BUT DID

NOT WORK WITH THE OTHER NITROGEN CONSTITUENTS. THEIR WORK FURTHER INDICATED THAT THERE WAS NO WATER REABSORPTION IN THE CLOACA. THEY FOUND IT WAS NECESSARY TO INCLUDE ONE PERCENT SALT IN THE RATION OF SURGICALLY MODIFIED HENS TO PREVENT DEHYDRATION. BIRDS NOT RECEIVING ONE PERCENT SALT HAD ALL THE SYMPTOMS OF DEHYDRATION (HEMO CONCENTRATION, LOSS OF BODY WEIGHT, INCREASED WATER CONSUMPTION, ETC.).

DIXON AND WILKINSON (1957) PUBLISHED A SURGICAL TECHNIQUE FOR THE EXTERIORIZATION OF THE URETERS WHICH WAS FOLLOWED BY GOOD LIVEABILITY OF THE MODIFIED BIRDS. MANY OF THESE BIRDS CONTINUED TO LAY AFTER SURGERY. THE DIET FED THE SURGICALLY MODIFIED HENS CONTAINED ONE PERCENT SALT.

DIXON (1958) INVESTIGATED URINARY WATER REABSORPTION IN THE CLOACA BY COMPARING THE WATER EXCRETION OF EACH BIRD BEFORE AND AFTER SURGICAL MODIFICATION. HE USED THE TWO TYPES OF SURGICAL MODIFICATIONS (EXTERIORIZED URETERS AND EXTERIORIZED RECTA) AND SHOWED THERE WAS NO WATER REABSORPTION FROM THE CLOACA UNDER THE CONDITIONS OF THE EXPERIMENT. THE FEED USED IN THE EXPERIMENTS CONTAINED ONE PERCENT SALT. THE NITROGEN BALANCE DATA FROM THISSTUDY IS REPORTED IN THE EXPERIMENTAL SECTION OF THIS DISSERTATION.

NEWBERNE ET AL. (1957) PUBLISHED A SURGICAL METHOD FOR THE SEPARATE COLLECTION OF FECES AND URINE IN GROWING CHICKENS. THE SURGICAL TECHNIQUE INVOLVED WAS THE EXTERIORIZATION OF THE URETERS. THE COLLECTION OF URINE WAS MADE IN A RUBBER BALLOON AND THE FECES WAS COLLECTED IN A PLASTIC BAG.

THIS METHOD OF COLLECTION WAS USED BY LAERDAL ET AL. (1957), IN STUDIES DESIGNED TO DEVELOP A DIRECT METHOD FOR THE DETERMINATION OF DIGESTIBILITY IN GROWING CHICKENS. THEY FOUND THE NITROGEN RETENTION ON A 35 PERCENT CASEIN PURIFIED DIET TO BE 32.1 PERCENT. THE ADDITION OF

1.5 PERCENT ARGININE HYDROCHLORIDE INCREASED THE NITROGEN RETENTION TO 40.8 PERCENT. THE SUBSTITUTION OF GELATIN FOR 10 PERCENT OF THE CASEIN INCREASED THE NITROGEN RETENTION TO THE HIGHEST LEVEL OBTAINED, 45.6 PERCENT. A DIET CONTAINING 35 PERCENT LIVER PROTEIN GAVE THEM A NITROGEN RETENTION OF 39.7 PERCENT AND ON A PRACTICAL 22 PERCENT PROTEIN RATION THE NITROGEN RETENTION WAS 38.9 PERCENT. THE ENDOGENOUS NITROGEN AND METABOLIC FECAL NITROGEN WERE ESTIMATED ON A NITROGEN FREE DIET. THE VALUE FOR ENDOGENOUS NITROGEN WAS 385 MG. PER KG. OF BODY WEIGHT AND THE VALUE FOR THE METABOLIC FECAL NITROGEN WAS 551 MG. PER KG. OF FEED CONSUMED.

ARIYOSHI AND MORIMOTO (1956) PUBLISHED A METHOD FOR THE SEPARATE COLLECTION OF URINE AND FECES FROM MATURE BIRDS USING AN EXTERIORIZED RECTUM OPERATION. THEY FOUND THAT A CANNULA INSERTED IN THE ANAL OPENING WOULD ALLOW NORMAL EXCRETION OF FECES FROM BIRDS FED A LOW-FIBER PURIFIED DIET OR SEMIPURIFIED DIET. THEIR DIETS CONTAINED ONE PERCENT SALT AND NO POLYURIA WAS OBSERVED. THEY APPLIED SOME QUALITATIVE CHEMICAL TESTS TO THE URINES COLLECTED BUT REPORTED NO QUANTITATIVE ANALYTICAL DATA FOR THE VARIOUS NITROGEN COMPONENTS.

ARIYOSHI (1957) REPORTED FURTHER ON THESE STUDIES IN WHICH HE ASSESSED THE PROTEIN REQUIREMENT FOR MAINTENANCE OF NAGOYA STRAIN COCKS AND CAPONS. HE CAME TO THE FOLLOWING CONCLUSIONS:

"(1) THE MINIMUM ENDOGENOUS NITROGEN IN THE URINE WAS 0.65 ± 0.05 MG. PER 0.75 POWER OF BODY WEIGHT IN GRAMS.

(2) ESTIMATION OF THE BIOLOGICAL VALUE OF FEED PROTEIN IS QUITE POSSIBLE, USING BIRDS WITH ARTIFICIAL ANI.

(3) THE BIOLOGICAL VALUE OF THE PROTEIN IN LABORATORY

PREPARED, DRIED, WHOLE EGG POWDER WAS ESTIMATED AS 100 PERCENT IN ADULT BIRDS.

(4) THE MOST FAVORABLE AMINO ACID BALANCE FOR THE MAINTENANCE OF ADULT BIRDS MAY BE THAT OF WHOLE EGG PROTEIN.

(5) THE PROTEIN REQUIREMENT FOR THE DAILY MAINTENANCE OF THE ADULT BIRDS WHICH HAVE LIGHTER BODY WEIGHTS THAN 2.3 KG. MAY BE LOWER THAN 1.8 GM. (AS IDEAL PROTEIN OF 100 PERCENT TRUE DIGESTIBILITY AND BIOLOGICAL VALUE)."

THESE COLLECTION TRIALS INVOLVED AS MANY AS THREE, EIGHT TO 14 DAY TEST PERIODS RUN SERIALLY IN WHICH LOW-PROTEIN AND NON-PROTEIN RATIONS WERE FED. A PERIOD OF ABOUT TWO MONTHS ON LOW-PROTEIN DIETS WAS REQUIRED FOR THE URINARY NITROGEN LEVEL TO REACH A UNIFORM MINIMUM. A CONSIDERABLE WEIGHT LOSS FOR SEVERAL OF THE BIRDS WAS EXPERIENCED DURING THIS SERIES OF TRIALS. AFTER A SERIES OF SEVEN COLLECTION PERIODS SEPARATED BY VARIOUS LENGTHS OF TIME, HE INCREASED THE PROTEIN ALLOWANCE AND OVER A PERIOD OF 43 DAYS SOME OF HIS BIRDS GAINED A GREAT DEAL OF WEIGHT (VARYING FROM 200 TO 1000 GMS. PER BIRD). A STUDY OF THE DATA PRESENTED IN THE PAPER SUGGESTS THAT THE LONG INTERIM ON LOW AND NON-PROTEIN RATIONS BROUGHT ABOUT THE ADAPTATION TO A LOW RATION PROTEIN LEVEL IN SOME BIRDS AND NOT IN OTHERS.

SHARPE, DAVIS, AND COULSON AND HUGHES (LOC. CIT.) REPORTED URIC ACID NITROGEN ACCOUNTED FOR THE MAJOR PERCENTAGE OF THE URINARY NITROGEN OF CHICKENS.

THE LIVER OF CHICKENS AND PIGEONS PRODUCE HYPOXANTHINE WHICH IS THEN OXIDIZED TO URIC ACID IN THE LIVERS OF CHICKENS AND KIDNEYS OF PIGEONS

(KREBS 1936). TEEKELL (1958) SHOWED THAT THE KIDNEYS OF CHICKENS EXHIBIT HIGH XANTHINE OXIDASE ACTIVITY.

ACCORDING TO THE COMBINED ISOTOPIC STUDIES OF SONNE ET AL. (1949), BUCHANAN (1951) AND ELWIN AND SPRINSON (1950); THE CARBONS OF URIC ACID COME FROM CO_2, GLYCINE, AND FORMATE AND THE NITROGEN COMES FROM THE METABOLIC POOL AND FROM GLYCINE.

SHEMIN AND RITTENBERG (1947) SHOWED THAT PURINE SYNTHESIS IN MAMMALS INVOLVED THE SAME SOURCE OF RAW MATERIALS. IT IS INTERESTING TO NOTE THAT IN MAMMALS AND IN THE AVIAN SPECIES THERE IS A SIMILAR SYSTEM FOR THE SYNTHESIS OF PURINES. IN BIRDS THE PURINE SYNTHESIS GIVES RISE TO THE CHIEF NITROGEN EXCRETORY PRODUCT, WHEREAS, IN MAMMALS THIS SYNTHESIS SEEMS TO BE USED AS A SOURCE OF PURINE BASES FOR THE FORMATION OF NUCLEIC ACIDS AND UREA IS THE CHIEF NITROGEN CONSTITUENT IN THE URINE UNDER "NORMAL" CONDITIONS OF PROTEIN INTAKE AND METABOLISM.

O'DELL ET AL. (1958), FOUND THAT INCREASING THE FREE ARGININE LEVELS IN DIETS FOR CHICKS BROUGHT ABOUT AN INCREASE IN THE EXCRETION OF UREA IN THE URINE. THEY WERE ATTEMPTING TO DETERMINE THE REASON FOR THE HIGHER ARGININE REQUIREMENT OF CHICKS ON PURIFIED CASEIN RATIONS AS COMPARED TO PRACTICAL RATIONS.

> "IT WAS POSTULATED THAT THE HIGHER REQUIREMENT FOR ARGININE ON AN ARGININE-SUPPLEMENTED CASEIN DIET THAN ON A PRACTICAL TYPE DIET IS DUE IN PART TO A MORE RAPID ABSORPTION OF THE FREE AMINO ACID AND CONSEQUENTLY A MORE RAPID BREAKDOWN TO UREA BY KIDNEY ARGINASE."

BELL, (1957) FOUND THAT 1.2 MG. PERCENT OF UREA NITROGEN IN

THE BLOOD OF NORMAL FOWLS. HE POINTS OUT VERY ADEQUATELY THAT THE SOURCE OF UREA IS UNKNOWN ALTHOUGH ARGINASE HAS BEEN FOUND IN THE KIDNEY. HE DETERMINED UREA BY THE CONWAY MICRODIFFUSION TECHNIQUE WHICH REQUIRES THE DETERMINATION OF AMMONIA ALSO. No AMMONIA WAS FOUND IN THE BLOOD.

THIS POSES AN INTERESTING PROBLEM FOR FUTURE STUDY. THE CURRENT THEORY IS THAT AMMONIA IS PRODUCED IN THE KIDNEY AND IS IMMEDIATELY EXCRETED. THE URINARY AMMONIA NITROGEN OF A NORMAL MAN IS ABOUT 0.7 GM. PER DAY. HIGH AMMONIA EXCRETIONS ARE USUALLY ASSOCIATED CLINICALLY WITH ACIDOSIS, (HAWK ET AL. 1954). IT IS INCREASED IN AMOUNT BY THE INGESTION OF ACID-FORMING FOODS AND IN THE ACIDOSIS OF STARVATION OR DIABETES (BUT NOT OF NEPHRITIS), AND DECREASED BY THE INGESTION OF ALKALIES OR BASE-FORMING FOODS AND IN ALKALOSIS. THIS IS A CASE INVOLVING A NITROGENOUS WASTE MATERIAL WHICH LEAVES THE KIDNEY FOR A PURPOSE AND SEEMINGLY LEAVES ONLY BY WAY OF THE URINE. IN CHICKENS IT IS POSTULATED THAT UREA IS FORMED IN THE KIDNEY ONLY, YET, IT IS FOUND IN THE BLOOD AT A LEVEL SIMILAR TO THAT OF URIC ACID IN MAN.

CREATINE AND CREATININE IN THE URINE OF MAMMALS COMES FROM THE CREATINE OF MUSCLE. (BLOCH AND SCHOENHEIMER 1940A AND 1940B).

THE URINARY AMINO ACIDS ARE "SPILL-OVERS" FROM THE BLOOD WHICH HAVE NOT BEEN DEAMINATED IN THE LIVER (DENT 1946).

SUMMARY

THERE IS CONSIDERABLE EVIDENCE IN THE LITERATURE THAT A CERTAIN PORTION OF THE URINARY NITROGEN EXCRETION REPRESENTS A NITROGEN METABOLISM WHICH IS CRITICAL FOR THE MAINTENANCE OF LIFE. THE URINARY NITROGEN COMPONENT WHICH IS MOST NEARLY REPRESENTATIVE OF THIS METABOLISM IS THE CREATININE EXCRETION IN MAMMALS AND POSSIBLY THE CREATINE EXCRETION IN AVIAN SPECIES.

THERE IS EVIDENCE ALSO THAT THE NITROGEN WITHIN THE ANIMAL IS IN A DYNAMIC STATE OF FLUX, I.E., THAT THE NITROGEN TAKEN IN TODAY IS NOT NECESSARILY EXCRETED TODAY.

THE PROBABLE SOURCES OF ALL THE NITROGEN METABOLITES PRESENT IN AVIAN URINE HAVE BEEN DETERMINED WITH THE POSSIBLE EXCEPTION OF UREA.

THERE HAS BEEN VERY LITTLE CONCLUSIVE WORK ON THE URINARY NITROGEN EXCRETION OF AVIAN SPECIES AND NONE ON THE EFFECT OF DIETARY REGIME UPON THE TOTAL NITROGEN COMPONENTS OF AVIAN URINE.

SECTION ONE

TECHNIQUES FOR THE QUANTITATIVE COLLECTION OF CHICKEN URINE AND FECES

Introduction

Studies with fowls involving separate collection of urine and feces have been quite limited. As a result, there is little published information on the surgical techniques and apparatus for such studies. The study of the nitrogen metabolism of the hen carried out in the experiments at the Louisiana Station necessitated a constant refinement of the surgical and collection techniques involved in the separate collection of feces and urine.

This section presents the information on these topics.

Surgical Techniques

The surgical techniques used in the studies reported in this dissertation were those developed by Dixon and Wilkinson (1957) and by Dixon (1958). These studies were begun as a collaboration with Dixon on the latter experiment. After the conclusion of the studies with Dixon certain refinements in surgical techniques were made in order to maintain long term collections from hens having exteriorized recta.

The chicken with an exteriorized rectum was the bird of choice for use in nutritional experiments. Their advantages compared to hens with exteriorized ureters were: (1) it was easier to collect excreta from them, (2) any nitrogenous excretion of the reproductive tract would be found in the urine, and (3) the egg could be deposited in a suitably constructed urine collection bottle without danger of breakage and

CONTAMINATION OF THE EXCRETA. THE PRIMARY DIFFICULTY ENCOUNTERED WITH EXTERIORIZED RECTUM TYPE OF MODIFICATION WAS THE FAILURE TO SECURE HEALING BETWEEN THE MUCOSA OF THE INTESTINE AND THE SKIN. ALTHOUGH SOME EXTERIORIZED RECTUM OPERATIONS SEEMED SUCCESSFUL FOR TWO TO FOUR WEEKS, EVENTUALLY THE SURROUNDING SKIN ENCROACHED ON THE ANAL OPENING AND THE ANUS BECAME SCABBED OVER. IN ONE CASE THE HEN WAS ALLOWED TO GO A WEEK AFTER SCAB FORMATION OCCURRED AND THE SKIN COMPLETELY COVERED THE ANAL OPENING. IT WAS APPARENT THAT SOME METHOD OF CANNULATION SIMILAR TO THAT USED BY ARIYOSHI AND MORIMATO (1956) WOULD HAVE TO BE DEVISED. CANNULAE SIMILAR TO HIS WERE TRIED BUT WOULD NOT REMAIN IN PLACE. A GLASS CANNULA WITH A WIDE RIM, HAVING FOUR HOLES FOR SUTURES, WAS DESIGNED. AFTER SEVERAL TRIALS, THE MOST SUITABLE SIZE WAS DETERMINED (FIGURE 1). IT WAS CONSTRUCTED FROM 10 M.M. GLASS TUBING WITH A LENGTH FROM THE RIM TO THE TIP OF 12 M.M. AND A DIAMETER ACROSS THE RIM OF 25 M.M. USING THIS CANNULA SUTURED IN PLACE AND A LOW FIBER DIET, LITTLE DIFFICULTY WAS ENCOUNTERED IN FECES COLLECTION.

THE BEST TIME FOR CANNULATION WAS AFTER A GREAT DEAL OF SCAR TISSUE HAD FORMED AROUND THE ANAL OPENING. THIS WAS USUALLY ABOUT THREE DAYS AFTER THE OPENING HAD FIRST SCABBED OVER. WHEN THE CANNULA WAS IN PLACE, THERE SEEMED TO BE A FORCE ATTEMPTING TO EXPEL IT. WHEN A HEN MANAGED TO EXPEL HER CANNULA, SHE BECAME CONSTIPATED. THE CANNULA SEEMED TO PROVIDE A CONSTANT IRRITATION OF THE RECTUM WHICH WAS NECESSARY FOR NORMAL EXCRETION. WHEN THE CANNULA WAS CORRECTLY SEATED, THE SPHINCTER MUSCLE CLOSED OVER THE INTERIOR OPENING OF THE CANNULA AND FECES WERE PASSED IN THE SAME MANNER AS THAT IN MANY OTHER SPECIES. THE FEED CONTAINED NO MORE THAN FIVE PERCENT FIBER, COMPOSED OF ALPHA-CEL, AGAR AND METHYL-CELLULOSE. IT WAS FOUND

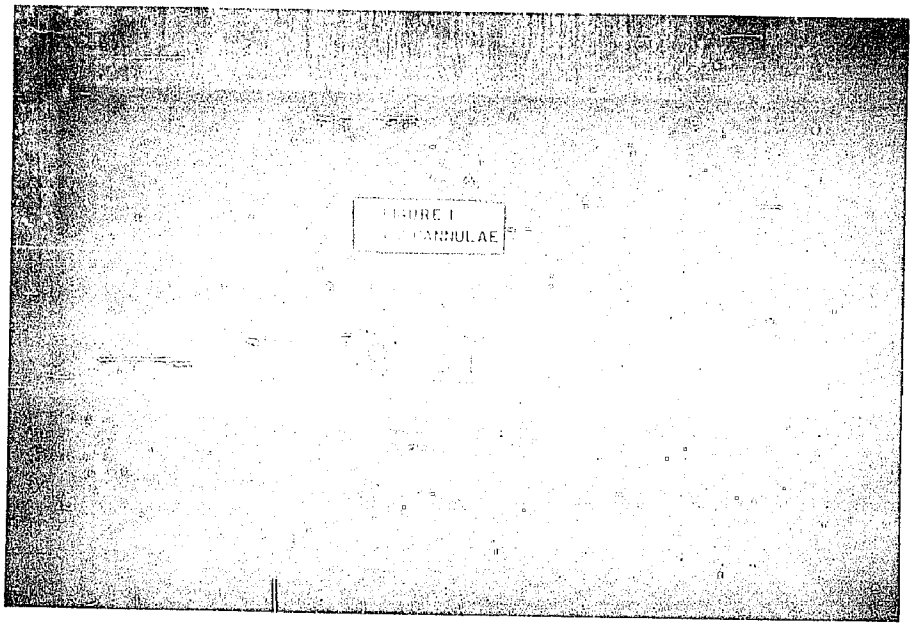
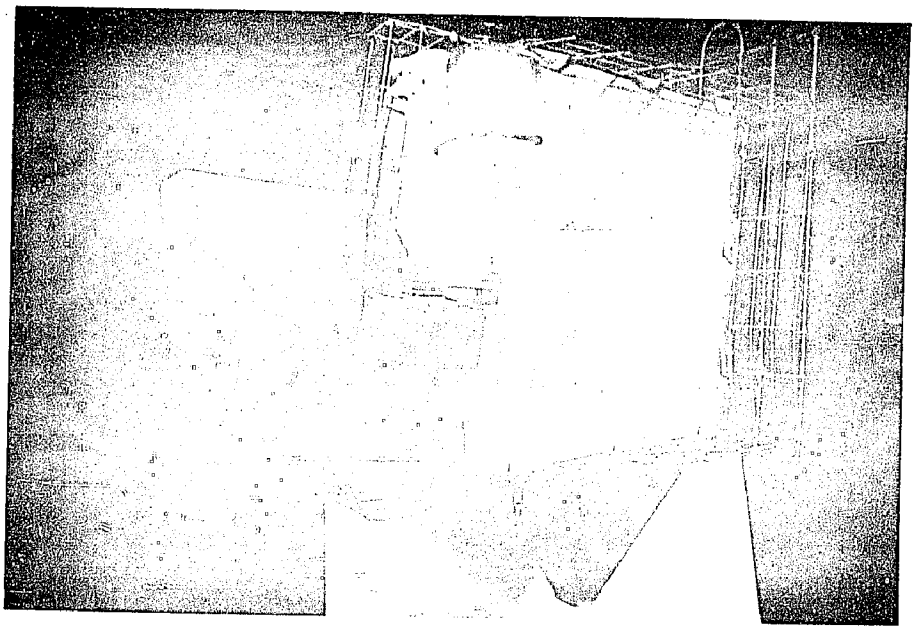


FIGURE 2

INDIVIDUAL METABOLISM CAGES



THAT EXCESS FIBER WOULD DRY IN THE CANNULAE, CLOG IT UP, AND RESULT IN EXPULSION OF THE CANNULA.

THE WHITE LEGHORN HENS USED IN THIS EXPERIMENT BEGAN LAYING UPON REGAINING THEIR WEIGHT AFTER THE SUCCESSFUL CANNULATION TECHNIQUE WAS DEVISED. THEY ARE PRESENTLY IN EXCELLENT HEALTH (FOUR TO EIGHT MONTHS AFTER CANNULATION) AND ARE LAYING.

Excreta Collection Techniques

IN TRIALS ONE AND TWO THE HENS WERE PLACED IN INDIVIDUAL METABOLISM CAGES FIVE INCHES WIDE X 20 INCHES LONG X 14 INCHES DEEP. THESE CAGES (FIGURE 2) HAD SLANTED WIRE FRONTS TO PREVENT EACH HEN FROM "STEALING" WATER OR FEED FROM HER NEIGHBOR. THE FEED TROUGH WAS MOUNTED ON THE CAGE FRONT AND THE WATER CUP ON THE LEFT SIDE OF THE CAGE FRONT. THE HENS COULD NOT MOVE AROUND IN THESE CAGES AND TUBES MADE FROM RUBBER ARTIFICIAL VAGINA LININGS CONDUCTED THE URINE FROM THE EXTERNAL URETERAL OPENINGS TO A 500 ML. GRADUATED CYLINDER MOUNTED ON THE BACK OF THE CAGE. THE WEIGHT OF THE TUBE WAS SUPPORTED BY "CHICKEN SWEATERS" MADE OF CLOTH IN SUCH A MANNER THAT THEY ENCLOSED THE MAJOR PORTION OF THE BODY AND WERE HELD IN PLACE BY THE WINGS AND LEGS. IT WAS FOUND NECESSARY TO SUTURE THE COLLECTION TUBE TO THE SKIN JUST BELOW THE URETERAL OPENINGS WHEN COLLECTING FROM HENS HAVING EXTERIORIZED URETERS BUT UNNECESSARY TO SUTURE THE TUBE IN PLACE WHEN COLLECTING FROM BIRDS HAVING AN EXTERIORIZED RECTUM.

THE APPARATUS USED FOR FECES COLLECTION WAS A LARGE FUNNEL WHICH FITTED UNDER THE METABOLISM CAGE. THE FUNNEL MOUTH WAS 10 INCHES IN DIAMETER TAPERING TO A 2.5 INCH OPENING AT THE LOWER END. A 500 ML.

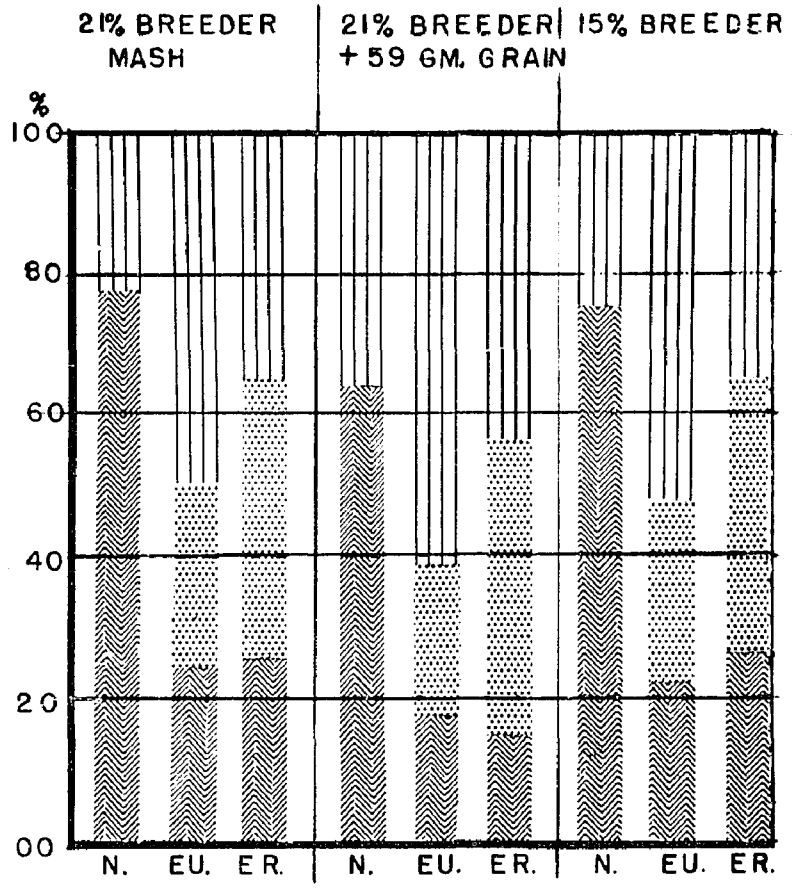
WARING BLENDOR JAR WAS ATTACHED TO THIS BY MEANS OF A WIRE BAIL EQUIPPED WITH HOOKS WHICH ENGAGED THE UPPER RIM OF THE FUNNEL. VERY LITTLE FECES STUCK IN THE FUNNEL AND THIS WAS SCRAPED DOWN INTO THE JAR WITH A SPATULA AT FOUR HOUR INTERVALS.

CONSTANT CHECKING ASSURED QUANTITATIVE URINE COLLECTION. THERE WAS ONE TROUBLESOME DETAIL, NAMELY; A YELLOW GRANULAR PRECIPITATE ACCUMULATED IN THE URINE COLLECTION TUBES AND THIS HAPPENED MORE OFTEN WITH THOSE HENS HAVING EXTERIORIZED URETERS THAN WITH THOSE HAVING EXTERIORIZED RECTA. THIS DIFFERENCE WAS NOT SURPRISING SINCE THE URINE CAME FROM THE EXTERIORIZED URETERS AS A STEADY OOZE BUT WAS EXPELLED WITH SOME FORCE FROM THE CLOACA OF THE HEN HAVING AN EXTERIORIZED RECTUM. THE PRESENCE OF PRECIPITATE IN THE COLLECTION TUBES MAY HAVE PREVENTED QUANTITATIVE RECOVERY OF URINE. WATER METABOLISM DATA WERE ALSO BEING COLLECTED AND THE TUBES COULD NOT BE WASHED BUT HAD TO BE SCRAPED WITH A SPATULA.

FIGURE 3 PRESENTS THE NITROGEN DATA ACCUMULATED IN TRIAL ONE. PROBABLY THE MOST STRIKING FACT IN THIS GRAPH IS THE DIFFERENCE IN THE APPARENT RESPONSE TO RATION TREATMENT AMONG THE THREE GROUPS OF HENS. NOTE THE APPARENTLY GREATER NITROGEN RETENTION OF THE SURGICALLY MODIFIED HENS. THERE WAS A MARKED DIFFERENCE BETWEEN THE HENS HAVING EXTERIORIZED URETERS AND THOSE HAVING EXTERIORIZED RECTA.

INSPECTION OF THE DATA SUGGESTED TWO HYPOTHESES: EITHER THE SURGICAL MODIFICATION HAD AFFECTED THE PROTEIN METABOLISM OR SOME URINARY NITROGEN WAS LOST DURING COLLECTION. IT WAS DOUBTFUL THAT THE SURGICAL TREATMENT WAS RIGOROUS ENOUGH TO SUPPORT THE FIRST HYPOTHESIS SO THE LATTER WAS ASSUMED TO BE TRUE. THIS MEANT THAT THE COLLECTION TECHNIQUE MUST BE

FIGURE 3
TRIAL ONE
EFFECT OF RATION ON N PARTITION



FURTHER REFINED TO ENSURE COMPLETE RETENTION OF URINARY NITROGEN.

ACCORDING TO DAVIS (1927) "NORMAL" HEN URINARY NITROGEN MAY BE PARTITIONED AS 17.3 PERCENT URIC ACID, 10.4 PERCENT UREA, 8.0 PERCENT CREATINE AND CREATININE, AND 1.4 PERCENT OF UNDETERMINED ORIGIN. IF THE URINES COLLECTED IN TRIAL ONE ARE CONSIDERED NORMAL, THE LOSS OF THE AMMONIA WOULD EXPLAIN THE DIFFERENCE BETWEEN THE BIRDS HAVING EXTERIORIZED RECTA AND THE NORMAL BIRDS. ANY EXPLANATION OF THE DIVERGENCE BETWEEN THE NORMAL BIRDS AND THOSE HAVING EXTERIORIZED URETERS MUST INVOLVE EITHER THE LOSS OF THE ADDITIONAL 10 PERCENT UREA NITROGEN OR SOME CHANGE IN NITROGEN METABOLISM.

IF THE AMMONIA WAS BEING LOST FROM THESE URINES (pH 6.25) IT SEEMED LOGICAL TO PLACE AN ACID PRESERVATIVE IN THE URINE RECEIVING VESSELS. IF THERE WAS A FURTHER LOSS OF NITROGEN FROM THE URINE OF THE HENS HAVING EXTERIORIZED URETERS IT MIGHT BE DUE TO THE BACTERIAL BREAKDOWN OF UREA. THIS FACTOR WOULD INFLUENCE THE CHOICE OF A PRESERVATIVE. ANOTHER FACTOR INVOLVED IN THE CHOICE OF A PRESERVATIVE WAS THE DESIRE TO STUDY THE NITROGEN CONSTITUENTS OF THE URINE IN THE FORM IN WHICH THEY WERE EXCRETED. A BRIEF STUDY OF URIC ACID CHEMISTRY SHOWED THAT THERE WAS A TENDENCY FOR URIC ACID TO DECOMPOSE IN EITHER ACID OR ALKALI. AT NEAR NEUTRAL pH IT IS QUITE STABLE.

BORIC ACID WAS CHOSEN AS THE ACID THAT MOST NEARLY SATISFIED THESE CONDITIONS. IT RAPIDLY COMPLEXES AMMONIA, IT IS HIGHLY ANTISEPTIC AT A CONCENTRATION OF ONE PERCENT, IT DOES NOT LOWER THE pH APPRECIABLY, IT COMPLEXES METALS WHICH MIGHT CATALYZE THE BREAKDOWN OF URINARY COMPONENTS, AND IT WILL NOT PRECIPITATE URIC ACID IN HARD GRANULES AS IS CHARACTERISTIC OF MANY OTHER ACIDS.

FIGURE 4
NITROGEN PARTITION AS AFFECTED BY PRESERVATIVE IN THE EXCRETA RECEIVING VESSELS

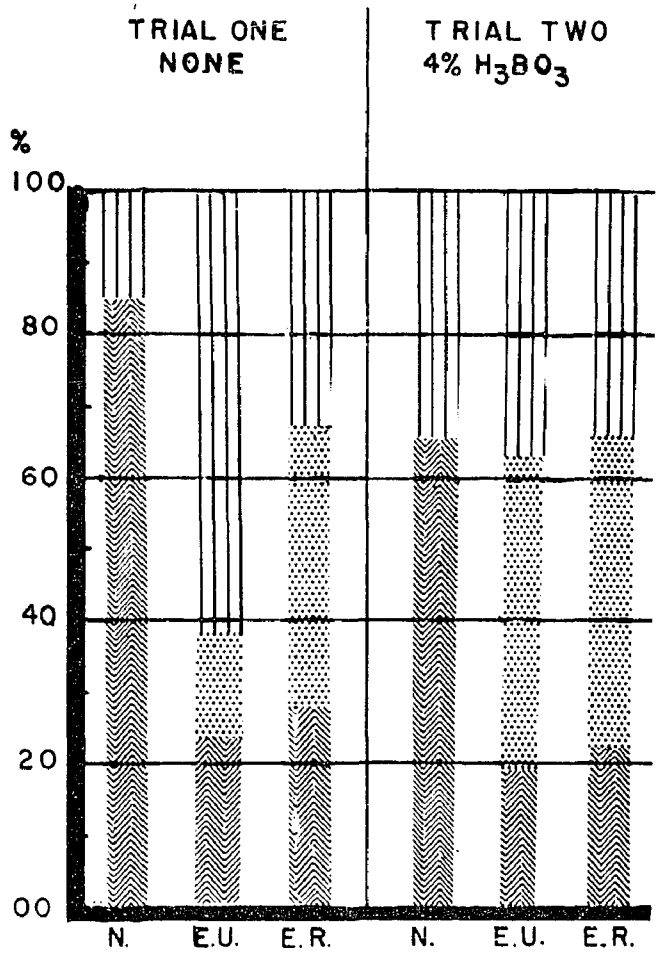


FIGURE 4 PRESENTS A COMPARISON OF THE DATA FROM TRIAL TWO AND TRIAL ONE. THE RATIONS FED WERE VERY SIMILAR BUT NOT IDENTICAL. NOTE THAT THE NITROGEN RETENTIONS BETWEEN THE VARIOUS SURGICAL TREATMENTS ARE NEARLY IDENTICAL IN TRIAL TWO. IN TRIAL TWO, 50 ML. OF FOUR PERCENT BORIC ACID WERE ADDED TO THE FECES AND URINE COLLECTION VESSELS WHEN THE URINE WAS COLLECTED. ASSUMING A FINAL VOLUME OF 200-250 ML. OF URINE, THE FINAL BORIC ACID CONTENT WAS ABOUT 0.8 - 1.0 PERCENT. THE FINAL VOLUME OF URINE WAS SELDOM MORE THAN 200 ML.

AS SHOWN BY THE NITROGEN ANALYSIS DATA (FIGURE 4), THIS METHOD OF COLLECTION OF EXCRETA WAS VERY SATISFACTORY. THERE WERE SOME TECHNICAL OBJECTIONS, NAMELY:

1. THE FOWL COULD NOT MOVE AROUND AS MUCH AS IN AN ORDINARY CAGE SO THIS METHOD OF COLLECTION MIGHT AFFECT CERTAIN PHASES OF METABOLISM.
2. ANIMALS HAD TO BE ADAPTED TO THE CLOSE CONFINEMENT FOR SEVERAL DAYS BEFORE NORMAL FEED CONSUMPTION COULD BE EXPECTED. IN SOME CASES THE ANIMAL NEVER BECAME ADAPTED.
3. AFTER ONE WEEK MANY HENS SHOWED SIGNS OF CAGE FATIGUE AND HAD TO BE REMOVED FROM THE EXPERIMENT.
4. THE URINE COLLECTION TUBES WERE DIFFICULT TO CLEAN AND MIGHT HAVE BEEN SOURCE OF ERROR IN OBTAINING QUANTITATIVE COLLECTIONS.

FOR THESE REASONS A NEW COLLECTION APPARATUS WAS DEVISED FOR HENS WHICH ALLOWED CONSIDERABLY MORE FREEDOM. THIS APPARATUS CONSISTED OF TWO PARTS; A HARNESS WHICH WAS FITTED TO THE HEN AND TWO POLYETHYLENE COLLECTING VESSELS.

THE HARNESS (FIGURE 5) CONSISTED OF A CLOTH KEEL PIECE AND A CLOTH AND WIRE BACK PIECE. THE KEEL PIECE WAS A DOUBLE THICKNESS OF CLOTH ABOUT THREE BY FIVE INCHES FOLDED LENGTHWISE. ONE END WAS CUT AND SEWED TO FIT THE FRONT OF THE KEEL. TIE STRINGS WERE SEWN IN SETS ALONG THE KEEL PIECE SO THAT THE FRONT PAIR FITTED IN FRONT OF THE WINGS, THE SECOND PAIR JUST BEHIND THE WINGS, THE THIRD PAIR JUST BEHIND THE LEGS, AND THE FOURTH PAIR RIGHT OVER THE END OF THE KEEL BONE. THE BACK PIECE WAS MADE OF CLOTH WITH A 12 GAUGE STEEL WIRE STIFFENER SEWN INTO THE PERIPHERY. SETS OF BUTTON HOLES WERE LOCATED JUST INSIDE THE WIRE IN SUCH A POSITION TO MATCH THE TIE STRINGS OF THE KEEL PIECE. THE STRINGS WERE BROUGHT UP OVER THE BACK AND THROUGH THE BUTTON HOLES AND TIED WITH SEVERAL KNOTS.

THE COLLECTION VESSELS (FIGURE 6) WERE CARVED FROM POLYETHYLENE BOTTLES TO FIT THE INDIVIDUAL HENS. THE URINE VESSELS WERE CARVED FROM EIGHT OUNCE BOTTLES AND THE FECES VESSELS FROM FOUR OUNCE BOTTLES. A BAFFLE WAS FITTED INTO THE URINE VESSELS TO PREVENT SPLASHING AND TO HOLD THE EGGS (FIGURE 7). THE BAFFLE WAS MADE FROM THE TOP OF AN EIGHT OUNCE BOTTLE AND WAS FITTED INTO PLACE BY FUSING THE PLASTIC WITH A SOLDERING GUN. THE VESSELS WERE FITTED WITH FOUR EYES MADE OF PAPER CLIP STEEL WIRE AND WERE HELD IN PLACE WITH LENGTHS OF UMBILICAL SUTURE FITTED WITH A STEEL CLIP AT ONE END AND A SAFETY PIN AT THE OTHER. ELASTIC WAS FIRST TRIED BUT THE SKIN OF THE HENS WAS SO TENDER THAT THERE WAS CONSTANT CHAFFING.

THE HARNESS DESCRIBED (FIGURE 8) WAS HELD IN PLACE SO RIGIDLY THAT THE HEN COULD NOT DISLODGE IT WHEN SHE SAT DOWN YET IT ALLOWED COMPLETE

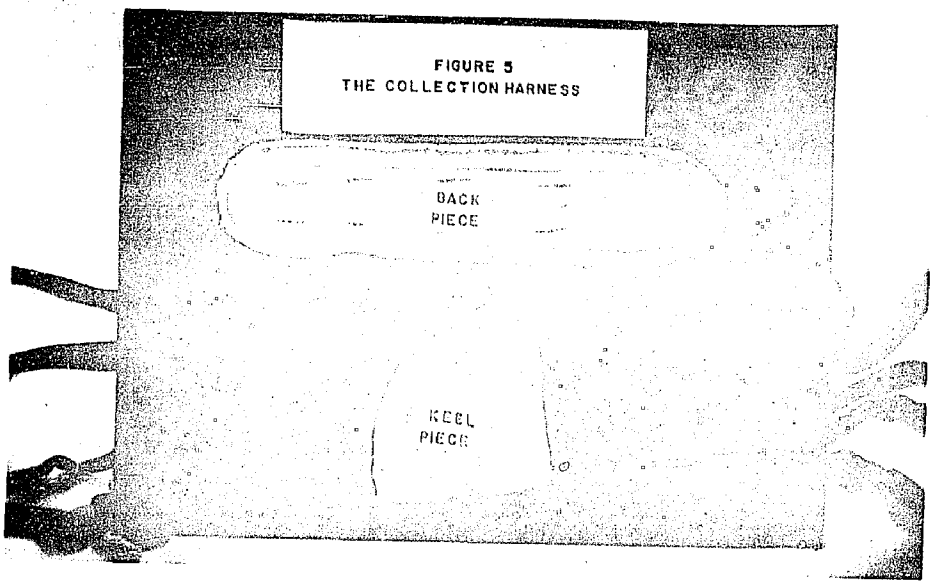


FIGURE 5
THE COLLECTION HARNESS

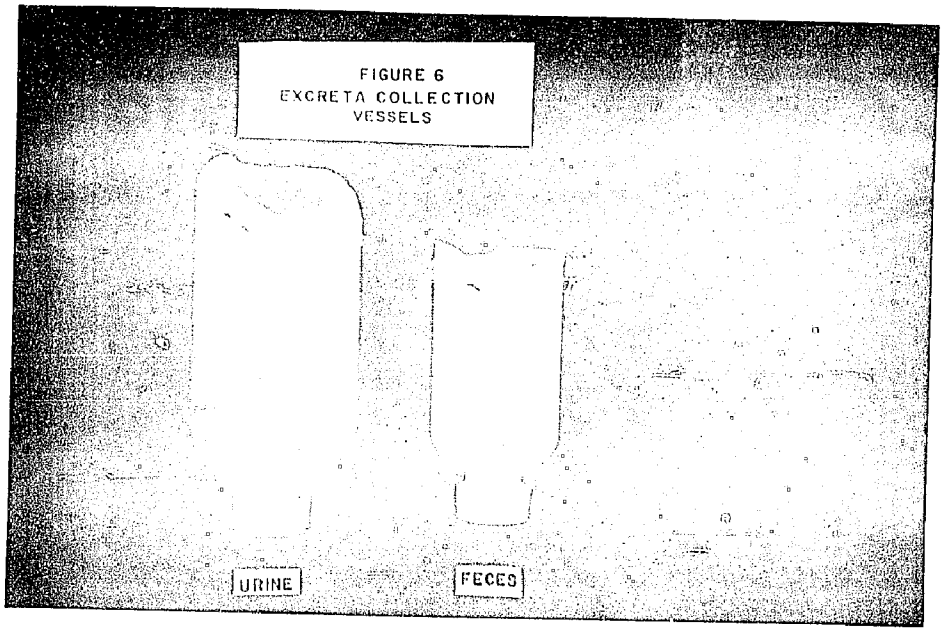


FIGURE 6
EXCRETA COLLECTION
VESSELS

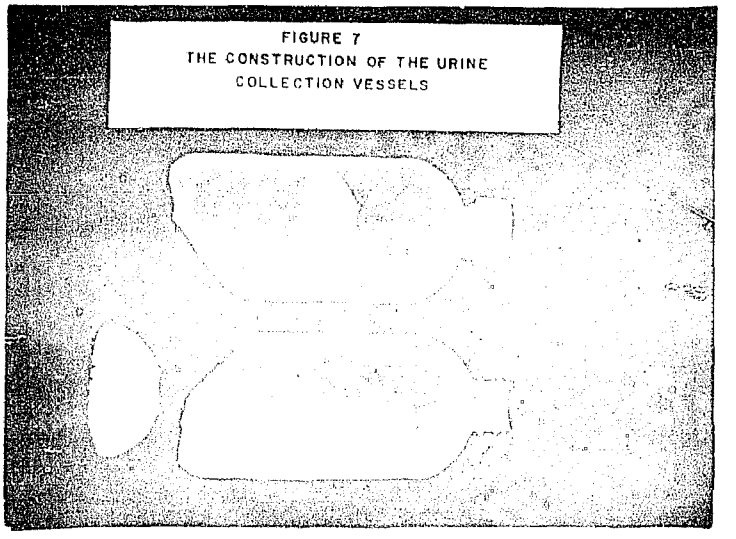


FIGURE 7
THE CONSTRUCTION OF THE URINE
COLLECTION VESSELS

FIGURE 8

SURGICALLY MODIFIED HEN WITH COLLECTION HARNESS IN PLACE

FREEDOM OF MOVEMENT. WITH THIS COLLECTION APPARATUS THE HENS WERE PLACED IN 1.5' x 2.5' INDIVIDUAL CAGES AND COLLECTIONS WERE MADE. THE URINE WAS COLLECTED TWO TIMES DAILY BY REMOVING THE CAP OF THE COLLECTION VESSEL AND DRAINING IT INTO A WIDE MOUTH GLASS JAR. WHEN RINSING WITH WATER WOULD NOT REMOVE ALL OF THE PRECIPITATE, THE BOTTLE WAS REPLACED AND QUANTITATIVE COLLECTION COMPLETED IN THE LABORATORY. THE DROPPING PANS UNDER EACH CAGE WAS COVERED WITH FREEZER PAPER, WAXED SIDE UP, SO THAT ANY URINE WHICH WAS SPILLED COULD BE RECOVERED WITH A PIPETTE. IN ONE OR TWO INSTANCES THIS PROVED TO BE A FORTUNATE SAFETY FACTOR. CERTAIN PLASTIC CAPS DID NOT SEAL THE COLLECTION BOTTLES AND LEAKS ALLOWED URINE TO DRIP ONTO THE PAPER. CAREFUL SELECTION AND TESTING OF CAPS PREVENTED THIS POTENTIAL ERROR.

Feeding Techniques with Purified Diets

THE USE OF PURIFIED DIETS OFFERED SOME DIFFICULTY. ONLY ONE HALF OF THE HENS ATE ENOUGH DRY MASH TO MAINTAIN THEIR WEIGHT. ARIYOSHI (1957) FED PURIFIED DIETS AS A WET MASH AND REPORTED GOOD CONSUMPTION. THE WHITE LEGHORN FEMALES USED IN THIS WORK OBJECTED TO WET MASH. EVEN THOSE WHICH CONSUMED ADEQUATE DRY FEED REFUSED WET FEED. ONE HEN WHICH CONSISTENTLY AND DELIBERATELY WET HER OWN FEED BEFORE EATING IT WOULD NOT EAT FEED THAT HAD BEEN WET BEFORE FEEDING. TO BRING ABOUT INCREASED CONSUMPTION A COMMERCIAL ADDITIVE CALLED "SUCRO FLAVOR" (FLAVOR CORP. OF AMERICA, CHICAGO, ILL.) WAS ADDED TO THE DIET AT A LEVEL OF 50 GRAMS PER 100 POUNDS OF FEED. ALTHOUGH THERE WAS SOME IMPROVEMENT IT WAS NOT SUFFICIENT WITH ALL THE HENS INVOLVED IN THE STUDY. IT WAS APPARENT THAT SOME OTHER METHOD WOULD HAVE TO BE FOUND TO FURTHER STIMULATE FEED CONSUMPTION ON THE PURIFIED DIET.

After a three month study with purified diets containing flavors, it was obvious that the physical form of the diet was influencing intake. The hens which readily ate the purified diet did as well as their sisters on a semi-purified diet in which the Drackett protein and zein were replaced with 50 percent protein soybean meal and part of the starch was replaced by corn meal. Various trials assured that this difference was not due to the fiber content. In any case the fiber content must be held at a low level for those hens fitted with cannulae. When the fiber levels fed these birds increased above five percent of the ration, the feces would harden in the cannulae and the birds would expel the cannulae. It was subsequently decided that the problem could be solved by using pelleted rations and a laboratory size pellet mill was secured (California Pellet Mill Co., San Francisco, Calif.).

Pelleting of Purified Diets

The first ration pelleted with the machine contained three percent alpha-cel and two percent agar as a source of bulk. This ration formed a very soft pellet which crumbled easily. About 80 ml. of water were added per pound of feed before pelleting and the wet pellets were dried in a forced draft oven at 60° C. for 12 hours. These pellets were not of desirable hardness and the drying was thought to be too rigorous. Methyl-cellulose was then substituted for the alpha-cel and the pellets were dried for 24 hours in a Jamesway incubator set at 38° C. with the water cut off to the humidifiers. The resulting pellets were hard and firm and decomposed very rapidly in water. Although many binders have been used with success in commercial pelleting operations, starch and cellulose are two of the more common ones. Methyl-cellulose was found to be very valuable in work with purified diets.

AGAR WAS ALSO PRESENT IN THESE DIETS AND MAY HAVE HAD AN ADDITIONAL BINDING EFFECT. THE FINAL DIET CONTAINED ABOUT 2.25 PERCENT AGAR, ONE PERCENT METHYL-CELLULOSE, 45 PERCENT CORN STARCH, AND 20 PERCENT CERELOSE PLUS THE MINERAL, VITAMIN AND PROTEIN SOURCES. HIGHER LEVELS OF CERELOSE RETARDED PELLET FORMATION.

THE PELLETS WERE VERY HARD AND WERE ABOUT ONE-EIGHTH INCH LONG. PELLETS FORMED FROM SEMIPURIFIED DIETS WERE FIRM BUT NOT SO HARD AS THOSE FORMED FROM PURIFIED DIETS. FEED WASTAGE WAS NEGLIGIBLE WHEN PELLETS WERE FED; CONVERSELY, THIS WAS A SERIOUS PROBLEM WITH MASH FEEDS.

IN PELLETING THESE FEEDS, IT WAS THOUGHT UNDESIRABLE TO INCLUDE COMMERCIAL BINDERS SUCH AS BENTONITE SINCE THE EFFECT OF SUCH MATERIALS ON DIGESTIBILITY HAD NEVER BEEN THOROUGHLY INVESTIGATED IN POULTRY NUTRITION. IT WAS THOUGHT THAT THE CARBOHYDRATE DERIVATIES, METHYL-CELLULOSE AND AGAR, WOULD HAVE LESS EFFECT UPON DIGESTIBILITY THAN SUCH MINERAL BINDERS.

SUMMARY

HENS WITH EXTERIORIZED RECTA COULD BE MAINTAINED IN AN EXCELLENT STATE OF HEALTH BY USING PROPERLY FITTED CANNULAE AND LOW FIBER DIETS.

A METHOD FOR THE QUANTITATIVE COLLECTION OF FECES AND URINE FROM HENS WITH EXTERIORIZED RECTA WAS DEVELOPED. THIS METHOD ALLOWED THE HEN COMPLETE FREEDOM TO MOVE AROUND IN A LARGE INDIVIDUAL CAGE.

THE CONSUMPTION OF PURIFIED DIET WAS IMPROVED BY ADDING AN ARTIFICIAL FLAVOR, HOWEVER, ADEQUATE CONSUMPTION WAS ACHIEVED ON PELLETED, PURIFIED RATIONS.

A METHOD OF PELLETING A PURIFIED DIET WAS DEVELOPED WHICH USED AN ELECTRIC POWERED, LABORATORY PELLET MILL.

METHYL-CELLULOSE PROVED TO BE A VERY GOOD BINDER FOR PURIFIED DIETS AT A LEVEL AS LOW AS ONE PERCENT OF THE DIET.

BECAUSE OF A RAPID EARLY LOSS OF CERTAIN NITROGEN CONSTITUENTS, IT WAS FOUND NECESSARY TO COLLECT THE FECES AND URINE IN THE PRESENCE OF A PRESERVATIVE AND BECAUSE OF CERTAIN CHARACTERISTICS BORIC ACID WAS THE PRESERVATIVE OF CHOICE.

SECTION TWO

THE EFFECT OF THE ENERGY LEVEL OF THE RATION UPON THE NITROGEN COMPONENTS OF THE URINE

Introduction

The interrelated metabolism of "energy nutrients" (carbohydrates and fats) and protein present a problem when one is formulating rations for the study of nitrogen metabolism. The effect of ration energy level upon the nitrogen components of avian urine had not been extensively studied. It was thought best to design a simple experiment to determine those effects before studying the effects of various protein levels upon the urinary nitrogen components.

This section of the dissertation deals with the results of this study.

Experimental Methods

The surgical techniques were those described by Dixon and Wilkinson (1957). The collection techniques were those developed by Dixon and previously described in Section One of this dissertation.

Twelve S. C. White Leghorn hens were selected and placed in an air conditioned room ($75^{\circ} \pm 1^{\circ}$ F.) in individual cages. The rations were fed ad libitum and water was before the birds at all times. Water and feed consumption were measured daily at 2:00 o'clock P.M.

The two rations fed in this study (Table 1) differed only in the substitutions of No. two animal tallow for rice hulls. These rations

TABLE 1

RATIONS FED IN TRIAL 2

INGREDIENT	RATION 15/900 PERCENT	RATION 15/800 PERCENT
SOYBEAN MEAL (50 PERCENT PROTEIN)	14.0	14.0
GROUND YELLOW CORN	50.5	50.0
GROUND OATS	8.5	8.5
ALFALFA MEAL	3.0	3.0
FISH MEAL	3.0	3.0
DI CAL.	5.0	5.0
GROUND OYSTER SHELL	4.0	4.0
SALT	1.0	1.0
NO. 2 TALLOW	5.2	0.0
RICE HULLS	4.8	10.0
VITAMIN PREMIX*	1.0	1.0
MANGANESE SULFATE	20 GMS.	20 GMS.

* THE VITAMIN SUPPLEMENT SUPPLIED THE FOLLOWING AMOUNT OF THE VARIOUS FACTORS PER POUND OF RATION.

NAME	
RIBOFLAVIN	2 MG.
CALCIUM PANTOTHENATE	4 MG.
NIACIN	12 MG.
CHOLINE CHLORIDE	76 MG.
VITAMIN B_{12}	6.2 MG.
PENICILLIN	2 MG.
D-L METHIONINE	1.0 MG.
VITAMIN A	3500 IU
VITAMIN D	400 ICU

WERE FORMULATED TO CONTAIN 15 PERCENT PROTEIN AND EITHER 900 CALORIES (RATION 15/900) OR 800 CALORIES OF PRODUCTIVE ENERGY PER POUND (RATION 15/800). IT WAS REALIZED IN THE BEGINNING THAT THIS WAS NOT A VERY RADICAL ENERGY TREATMENT BUT IT WAS BELIEVED THAT THERE SHOULD BE SOME DIFFERENCE IN RESPONSE IF THE POSTULATED INTERRELATIONSHIP OF PROTEIN AND ENERGY WERE OF TRUE METABOLIC ORIGIN.

THE HENS WERE PLACED ON RATION 15/900 FOR ONE WEEK AND COLLECTIONS WERE MADE OF THEIR NORMAL EXCRETA FOR A PERIOD OF THREE DAYS WITH 50 MLS. OF FOUR PERCENT BORIC ACID USED AS A PRESERVATIVE. THE HENS WERE THEN ALLOWED A WEEK TO ADJUST TO RATION 15/800 AND EXCRETA COLLECTIONS WERE MADE AGAIN FOR A THREE DAY PERIOD.

THE HENS WERE RETURNED TO RATION 15/900 AND EIGHT BIRDS WERE SURGICALLY MODIFIED SO THAT FOUR HENS HAD EXTERIORIZED URETERS AND FOUR HAD EXTERIORIZED RECTA. FOUR NORMAL HENS WERE KEPT AS CONTROLS. THE SURGICALLY MODIFIED HENS WERE COMPLETELY HEALED AFTER A WEEK AND COLLECTIONS WERE MADE IN THE SAME MANNER AS DURING THE NORMAL PERIOD.

THE TOTAL NITROGEN OF FECES, FEED AND URINE WAS DETERMINED BY THE KJELDAHL METHOD USING A COPPER CATALYST. THE URIC ACID NITROGEN WAS DETERMINED BY THE METHOD OF LAERDAL ET AL. (1957) WITH THE EXCEPTION THAT ONLY A 20 ML. ALIQUOT OF URINE WAS USED. VARIOUS COLORIMETRIC PROCEDURES FOR THE DETERMINATION OF URIC ACID WERE TRIED BUT THESE METHODS GAVE URIC ACID NITROGEN VALUES GREATLY IN EXCESS OF THE TOTAL NITROGEN PRESENT WITH THE EXCEPTION OF THE SILVER LACTATE PRECIPITATION METHOD OF FOLIN AS PRESENTED BY HAWK ET AL. (1954) WHICH GAVE A LOW VALUE WITH URINE AND STANDARDS.

The ammonia and urea nitrogen were determined by the Van Slyke and Cullen aeration procedure as presented by Hawk et al. (1954), with the exception that the aeration was done on a special apparatus which allowed simultaneous determinations. The apparatus and method appear in the Appendix Section II along with the procedure for the determination of creatine and amino acid nitrogen.

The creatine nitrogen was determined by a procedure based on the Jaffe reaction. The method for the determination of the amino acid nitrogen combined an ion-exchange de-salting technique, (Awapara and Sato (1956)), to remove urea and ammonia, and a ninhydrin colorimetric procedure (Meyer, (1957)) for the quantitative determination of amino nitrogen.

The amino acids were qualitatively separated and identified by paper chromatography. The uni-dimensional chromatograms were run using a 50:10:40 mixture of n-butanol, acetic acid and water. Two dimensional chromatograms were run using a 50:10:40 mixture of butanol, collidine and water following the acid solvent. In each case the lower or aqueous phase was discarded. The location of the spots was determined by dipping the paper in a solution of 0.2 percent ninhydrin in ethanol containing 1.0 percent pyridine. The chromatograms were dried in an infrared oven.

Results and Discussion

The response of the normal hens to the ration treatment is represented in Figure 9. It will be noted that nitrogen intake and retention was greater on the 900 Calorie diet than on the 800 Calorie diet. The nitrogen excretion was greater on the low-energy diet. Figure 10 represents the response of the surgically modified hens. Note that the response is

FIGURE 9
TRIAL TWO
SUMMATION OF RATION EFFECTS
(NORMAL HENS)

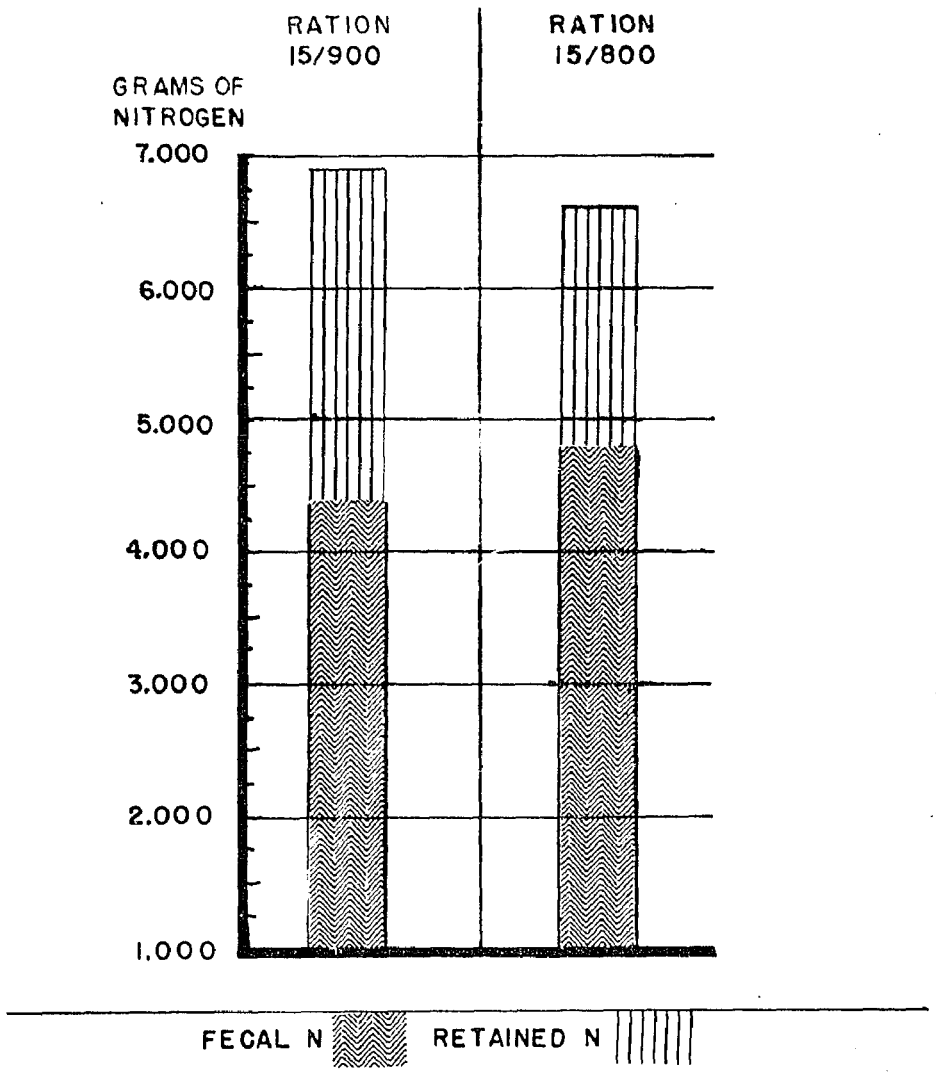


FIGURE 10
TRIAL TWO
SUMMATION OF RATION EFFECTS
(SURGICALLY MODIFIED HENS)

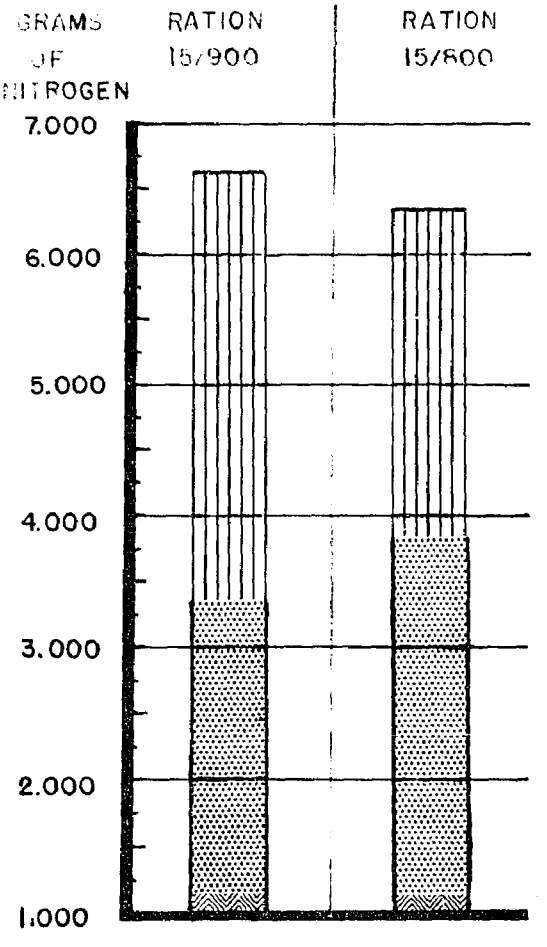


TABLE 2

NITROGEN ANALYSIS DATA, TRIAL 2

Hen No.	Ration No.	Nitrogen Intake[1] Mgms.	Fecal Nitrogen[1] Mgms.	Milligrams of Urinary Nitrogen						Percentage Composition of Urinary Nitrogen					
				Total	NH_3	Urea	Uric Acid	Amino Acid	Creat.[2]	NH_3	Urea	Uric Acid	Amino Acid	Creat.[2]	Unknown
9*	15/900	5977	1564	2157	503	301	1333	24	21	23	14	62	1.0	1.0	00
9*	15/800	2788	534	1785	168	65	783	56	25	9	4	44	3.0	1.4	38.6
12	15/900	7483	1412	3199	839	141	2149	33	19	26	4	67	1.0	0.6	1.4
17	15/900	5717	954	3120	334	32	2543	36	20	11	1	82	1.0	0.6	4.4
Average		6600	1183	3160	586	86	2346	35	20	18	2	74	1.0	0.6	2.9
12	15/800	7197	1221	3432	442	202	2550	27	17	13	6	74	0.7	0.5	5.8
17	15/800	5403	1152	3846	49	327	3210	28	18	1	9	84	0.7	0.5	4.8
Average		6300	1186	3639	246	265	2880	28	18	7	8	79	0.7	0.5	5.3

* Not included in averages.

1 Total for three day period.

2 Total creatine and creatinine nitrogen.

ESSENTIALLY THE SAME FOR THE TWO SURGICALLY MODIFIED HENS AS FOR NORMAL HENS.

OF THE EIGHT SURGICALLY MODIFIED HENS STARTED ONLY THREE COMPLETED THE EXPERIMENT. THE FIVE LOSSES WERE DUE TO INFECTION FOLLOWING SURGERY (TWO HENS) AND TO THE HENS PECKING THE WOUNDS AFTER HEALING HAD BEGUN (THREE HENS). THE DATA WAS CONSIDERED VALID FROM ONLY TWO OF THE HENS COMPLETING THE EXPERIMENT. THE OTHER HEN HAD LOST SO MUCH OF HER BODY WEIGHT THAT SHE COULD HARDLY BE CONSIDERED NORMAL. AN INSPECTION OF TABLE TWO WILL SHOW, HOWEVER, THAT IN MANY RESPECTS SHE RESPONDED IN THE SAME MANNER AS THE OTHER TWO HENS.

THE DATA FROM THE QUANTITATIVE DETERMINATIONS OF THE URINARY NITROGEN COMPONENTS ARE SHOWN IN FIGURES 11 THROUGH 16. THE URIC ACID NITROGEN EXCRETION WAS LOWER ON THE HIGH-ENERGY DIET WHEN EXPRESSED BOTH AS ACTUAL AMOUNTS AND AS A PERCENTAGE OF THE URINARY NITROGEN. THERE WAS MORE AMMONIA NITROGEN EXCRETED ON THE HIGH ENERGY DIET WHILE THE UREA NITROGEN EXCRETED WAS GREATER ON THE LOW-ENERGY DIET.

THE AMINO ACID NITROGEN EXCRETED WAS VERY LOW. A COMPARISON OF FIGURE 14 AND FIGURE 9 SEEMS TO INDICATE THAT THE AMINO ACID NITROGEN LEVEL IN THE URINE WAS RELATED TO PROTEIN INTAKE. ALANINE, GLYCINE, GLUTAMIC ACID, CYSTINE, ARGININE, AND ASPARTIC ACID WERE PRESENT IN ALL URINE SAMPLES. HISTIDINE, ORNITHINE, LYSINE, TAURINE, AND CYSTEINE WERE ALSO PRESENT IN AT LEAST TWO URINES WHILE TYROSINE, HYDROXYPROLINE, SERINE, AND LEUCINE WERE PRESENT IN ONE URINE IN ADDITION TO ALL AMINO ACIDS LISTED ABOVE. THE PARTICULAR URINE SAMPLE CONTAINING ALL THE LISTED AMINO ACIDS WAS FROM A HEN WHICH LITERALLY GORGED HERSELF ON THE HIGH ENERGY DIET BUT UNFORTUNATELY WAS LOST BEFORE THE COLLECTIONS COULD BE COMPLETED.

FIGURE II

EFFECT OF ENERGY LEVEL ON URIC ACID NITROGEN EXCRETION

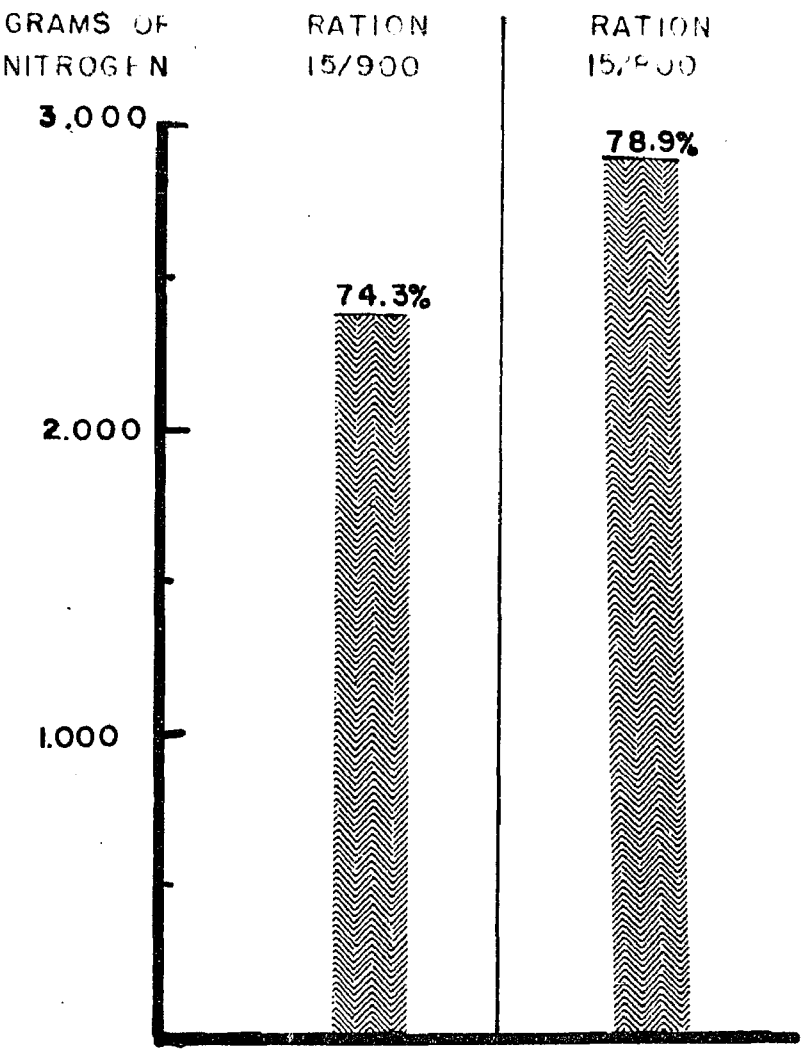


FIGURE 12
EFFECT OF ENERGY LEVEL ON AMMONIA NITROGEN EXCRETION

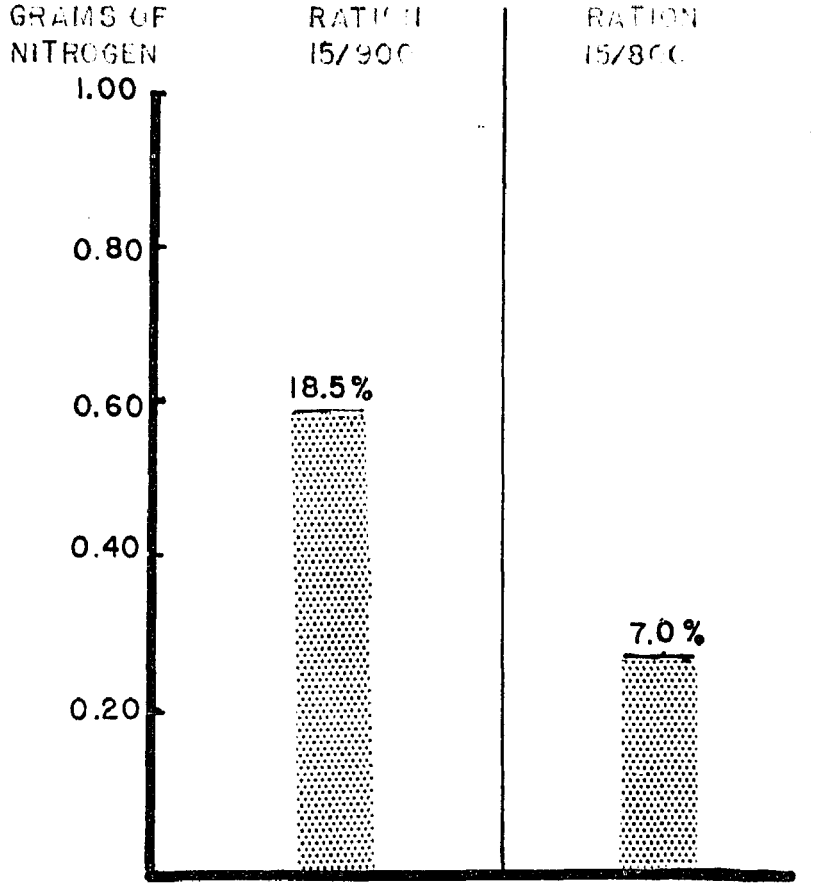


FIGURE 13
EFFECT OF ENERGY LEVEL ON UREA NITROGEN EXCRETION

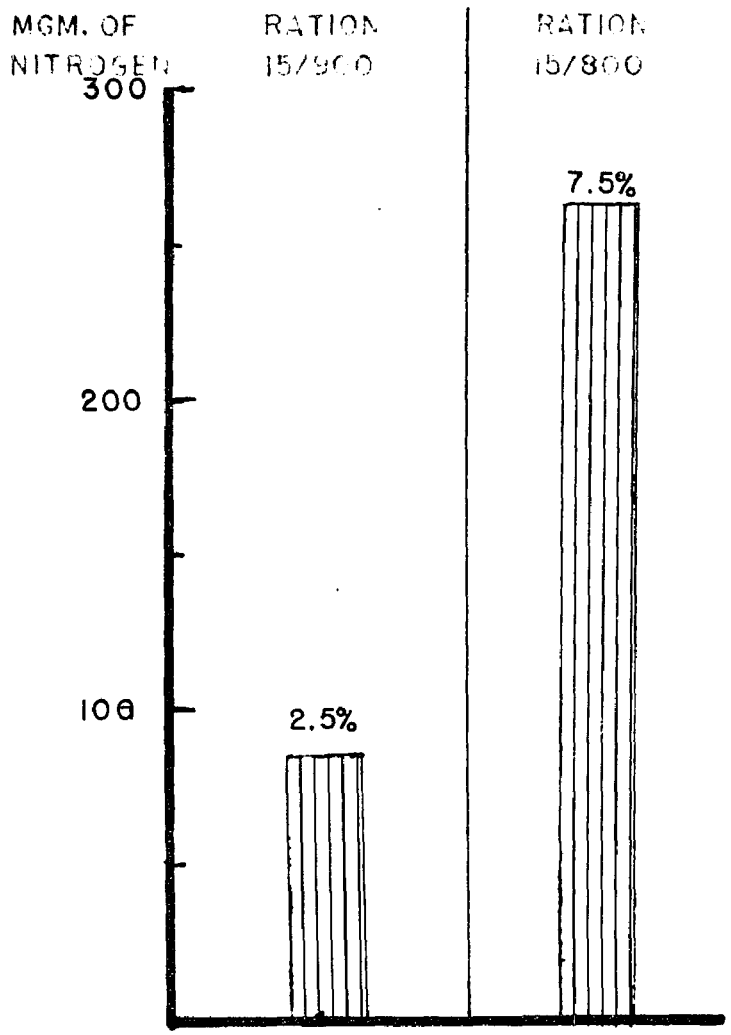
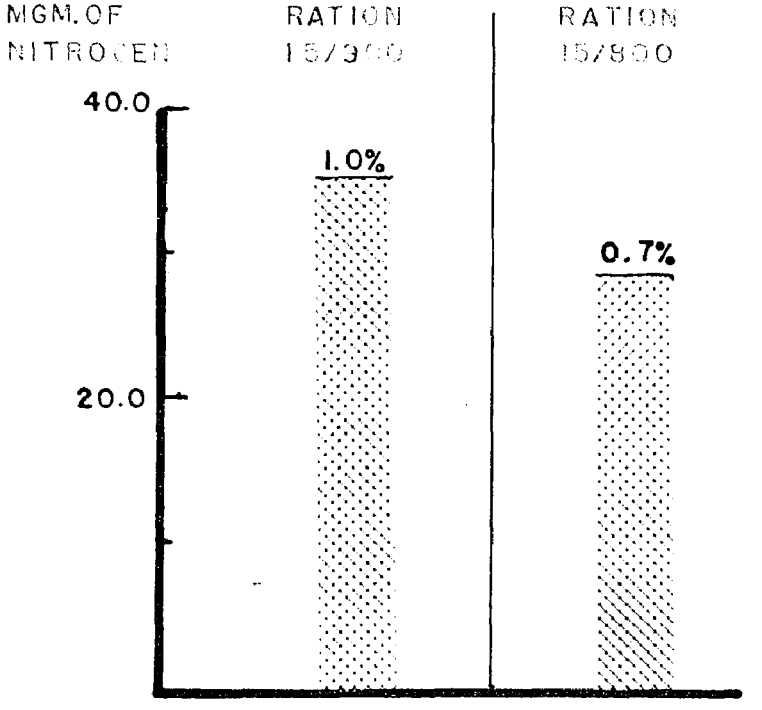


FIGURE 14
EFFECT OF ENERGY LEVEL ON ALPHA AMINO NITROGEN EXCRETION



QUALITATIVE AMINO ACID DETN.

ALANINE	HISTIDINE	TYROSINE
GLYCINE	ORNITHINE	HYDROXYPROLINE
GLUTAMIC A.	LYSINE	SERINE
CYSTINE	TAURINE	CYSTEINE
ARGININE	ASPARTIC A.	LEUCINE

FIGURE 15
EFFECT OF ENERGY LEVEL ON CREATINE NITROGEN EXCRETION

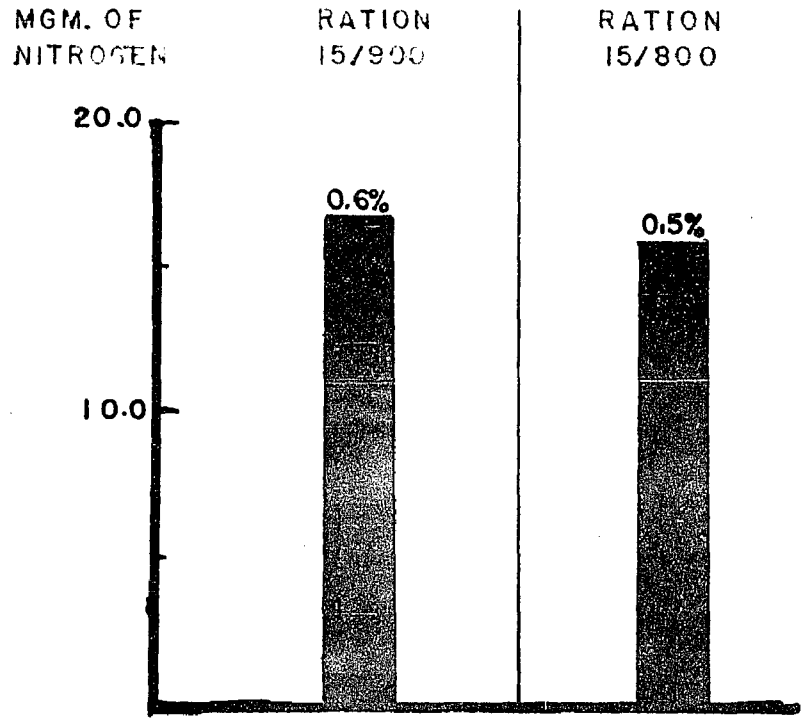
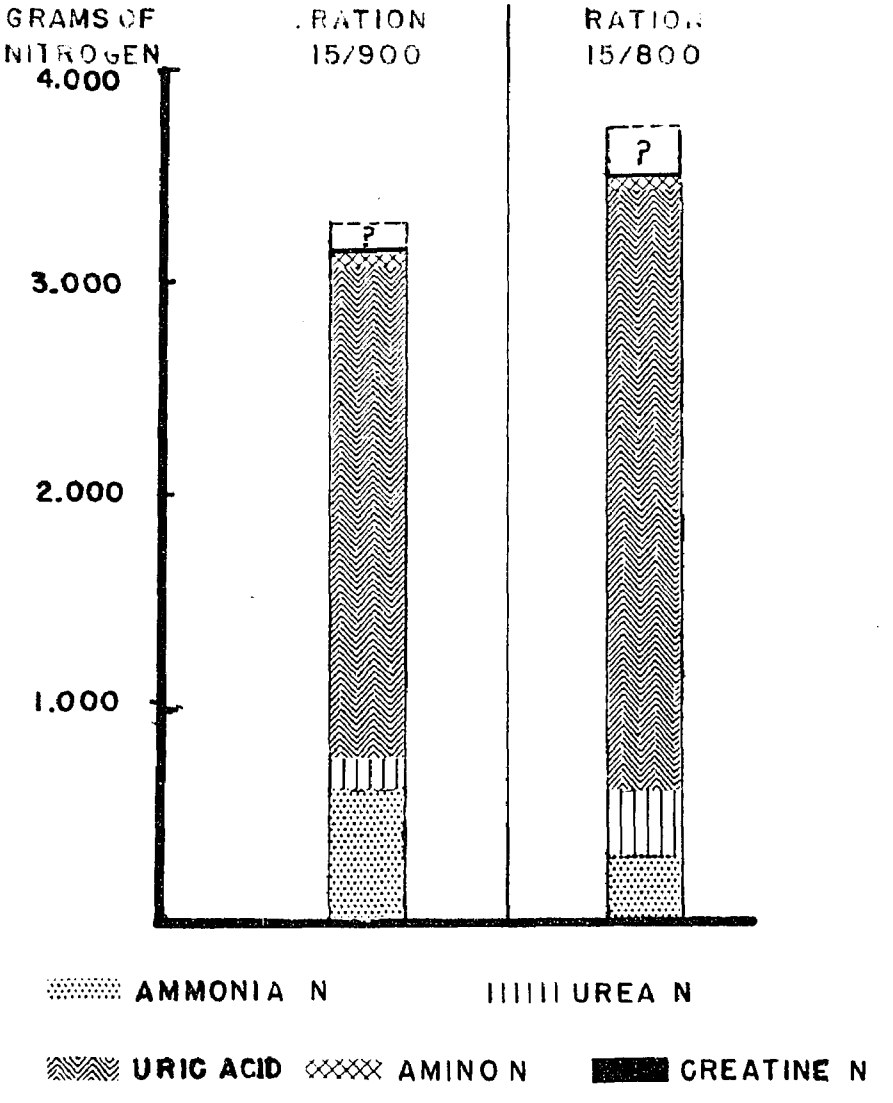


FIGURE 16
EFFECT OF ENERGY LEVEL ON THE
NITROGEN COMPONENTS OF THE URINE



THE CREATINE EXCRETION (FIGURE 15) REPRESENTS THE TOTAL CREATINE PLUS CREATININE. THE CREATININE NITROGEN EXCRETION WAS SO LOW AS TO BE INSIGNIFICANT (1.6 TO 2.9 MG. IN THREE DAYS).

THE EVIDENCE COLLECTED DURING THIS TRIAL INDICATED THAT THE SURGICAL MODIFICATION OF THE HENS INVOLVED DID NOT AFFECT THEIR NITROGEN METABOLISM. THE SURGICALLY MODIFIED HENS USED THEIR NITROGEN AS WELL AFTER SURGICAL MODIFICATION AS THEY DID BEFORE.

AS WAS EXPECTED, THE CREATINE NITROGEN WAS RELATIVELY CONSTANT. THE LACK OF NUMBERS LIMITED THE CONCLUSION BUT CREATINE NITROGEN EXCRETION MAY BE A BIOLOGICAL CONSTANT IN AVIAN SPECIES.

THE ONLY HYPOTHESIS WHICH CAN PRESENTLY BE OFFERED FOR THE IMPROVED UTILIZATION OF NITROGEN ON THE HIGH-ENERGY DIET IS THAT THE ADDITIONAL ENERGY BROUGHT ABOUT A DECREASE IN THE AMOUNT OF AMINO ACIDS DEAMINATED AND USED FOR ENERGY. THE CHANGE IN THE LEVELS OF AMMONIA, URIC ACID, AND UREA NITROGEN LEVELS IN THE URINE WERE, THEREFORE, REFLECTIONS OF THE AMOUNT OF AMINO ACIDS WHICH ENTERED DIFFERENT METABOLIC PATHWAYS.

THE INCREASED EXCRETION OF AMMONIA NITROGEN ON THE HIGH-ENERGY DIET MIGHT MEAN A CONDITION OF ACIDOSIS ON THAT RATION. NO TITRATABLE ACIDITIES OF THE URINES WERE RUN AND ONE CANNOT BE POSITIVE. HOWEVER, IF ONE EXAMINES THE TABLES OF FOLIN (1905B) HE WILL FIND THAT INCREASED AMMONIA NITROGEN EXCRETION WAS NOT NECESSARILY CONCOMITANT WITH AN INCREASE IN TITRATABLE ACIDITY. IT SEEMED DOUBTFUL THAT THE CHANGE IN ENERGY LEVELS BETWEEN THE TWO DIETS EMPLOYED IN THIS STUDY WOULD CHANGE THE TITRATABLE ACIDITY APPRECIABLY.

IT WOULD SEEM, THEREFORE, THAT THE CHANGES IN THE LEVELS OF THE URINARY NITROGEN COMPONENTS MUST COME FROM A CHANGE IN THE MAJOR SITES OF NITROGEN CATABOLISM RATHER THAN FROM A CHANGE OF PHYSIOLOGICAL CONDITION AS REFLECTED IN ACIDOSIS. SUCH AN HYPOTHESIS MUST BE BASED UPON KNOWN ENZYME SYSTEMS

FUNCTIONING IN THE BODY OF THE VERTEBRATES. ENZYME SYSTEMS INVOLVED IN NITROGEN METABOLISM ARE KNOWN TO EXIST IN QUANTITY IN THE LIVER AND KIDNEY. AS A WHOLE, THESE ENZYME SYSTEMS FOLLOW THE SAME METABOLIC PATTERN, JUDGING FROM THE ANALYSES OF "NORMAL" HUMAN URINE. THEIR EFFECTS ON THE NITROGEN COMPONENTS OF URINE, HOWEVER, SEEM TO INDICATE POSSIBLE DIVERGENCE IN THIS INSTANCE.

LOTSPEICH AND PITTS (1947) REPORTED THAT CERTAIN AMINO ACIDS WERE OXIDATIVELY DEAMINATED IN THE KIDNEY AND THE AMMONIA EXCRETED IN THE URINE. THE AMOUNT OF AMMONIA EXCRETED WAS DETERMINED TO A LARGE EXTENT BY THE CONCENTRATION OF AMINO ACID NITROGEN IN THE BLOOD.

IF, THEREFORE, THE AMINO ACIDS FROM THE HIGH-ENERGY DIET WERE SPARED DURING THE METABOLISM OF THE LIVER BECAUSE THE RATION ENERGY LEVEL WAS HIGH ENOUGH TO REDUCE THE USE OF THE AMINO ACID CARBON CHAIN FOR ENERGY, THE AMINO ACID LEVEL OF THE BLOOD COULD BE SO HIGH THAT THE AMINO ACIDS WOULD BE OXIDATIVELY DEAMINATED IN THE KIDNEY AND THE AMMONIA WOULD BE EXCRETED. IF THE AMMONIA WERE FORMED IN THE LIVER IT SHOULD BE EXCRETED AS URIC ACID OR POSSIBLY XANTHINE. THIS WOULD EXPLAIN THE INCREASED AMMONIA NITROGEN, THE DECREASED URIC ACID NITROGEN, AND THE INCREASED AMINO ACID NITROGEN EXCRETION ON THE HIGH-ENERGY DIET AS COMPARED TO THE LOW-ENERGY DIET. THE SIMPLE EXPLANATION WOULD BE THAT THE NITROGEN EXCRETED AS AMMONIA WAS AN EXCESS METABOLITE WHICH WAS ELIMINATED IN THIS MANNER. THE CONCENTRATION OF ENERGY METABOLITES IN THE LIVER FAVORED THE RETENTION OF THIS NITROGEN AS CIRCULATING AMINO ACIDS AND LESS OF THE EXCESS NITROGEN WAS INCORPORATED INTO HYPOXANTHINE THUS REDUCING URIC ACID EXCRETION.

AT PRESENT IT SEEMS IMPRACTICAL TO HYPOTHESIZE AS TO THE ORIGIN AND

REASON FOR THE INCREASED EXCRETION OF UREA NITROGEN ON THE LOW-ENERGY DIET. IF ALL OF THE DIETARY ARGININE WAS ACTED ON BY KIDNEY ARGINASE, THERE WOULD HAVE BEEN ABOUT 400 MG. OF UREA NITROGEN IN THE URINE. THIS DIET CONTAINED ABOUT 0.86 PERCENT ARGININE AS CALCULATED FROM AVERAGE ANALYSES. THIS WOULD MEAN THAT 50 TO 75 PERCENT OF THE GUANIDINO NITROGEN FROM THE DIETARY ARGININE WAS EXCRETED AS UREA WHEN THE HENS WERE FED A DIET WITH A MARGINAL ARGININE DEFICIENCY. ALTHOUGH THIS IS NOT IMPOSSIBLE, IT DOES SEEM IMPROBABLE.

SUMMARY

HENS RETAINED MORE DIETARY NITROGEN FROM A DIET CONTAINING 15 PERCENT PROTEIN AND 900 CALORIES PER POUND OF PRODUCTIVE ENERGY THAN FROM A DIET CONTAINING 15 PERCENT PROTEIN AND 800 CALORIES PER POUND OF PRODUCTIVE ENERGY. THIS RESPONSE WAS SHOWN WITH NORMAL HENS AND SURGICALLY MODIFIED HENS AND DEMONSTRATES THAT SURGICAL MODIFICATION DOES NOT INTERFERE WITH NITROGEN METABOLISM.

INCREASED ENERGY LEVEL IN ISO-PROTEIN RATIONS BROUGHT ABOUT AN INCREASE IN THE AMOUNT OF AMMONIA NITROGEN EXCRETED, A DECREASE IN THE AMOUNT OF URIC ACID EXCRETED, A DECREASE IN THE AMOUNT OF UREA NITROGEN EXCRETED, AND AN INCREASE IN THE AMOUNT OF AMINO NITROGEN EXCRETED. CREATINE EXCRETION WAS RELATIVELY CONSTANT FOR A GIVEN HEN AND WAS POSSIBLY AS MUCH A BIOLOGICAL CONSTANT AS IS CREATININE EXCRETION IN MAMMALS.

THE AMINO ACIDS PRESENT IN THE URINES WERE DETERMINED BY SINGLE AND TWO DIMENSIONABLE CHROMOTOGRAPHY. IT WAS FOUND THAT ALANINE, GLYCINE, GLUTAMIC ACID, CYSTINE, ARGININE, AND ASPARTIC ACID WERE PRESENT IN ALL

URINE SAMPLES. HISTIDINE, ORNITHINE, LYSINE, TAURINE, AND CYSTEINE WERE ALSO PRESENT IN AT LEAST TWO URINES WHILE TYROSINE, HYDROXYPROLINE, SERINE, AND LEUCINE WERE PRESENT IN ONE URINE IN ADDITION TO ALL AMINO ACIDS LISTED ABOVE. THE PARTICULAR URINE SAMPLE CONTAINING ALL THE LISTED AMINO ACIDS WAS FROM A HEN WHICH LITERALLY GORGED HERSELF ON THE HIGH ENERGY DIET.

IT IS HYPOTHESIZED THAT THE INCREASED ENERGY LEVELS CAUSED THE NITROGEN TO FOLLOW CERTAIN METABOLIC PATHWAYS AT THE EXPENSE OF OTHERS.

SECTION THREE

THE EFFECT OF DIETARY PROTEIN LEVEL UPON THE URINARY NITROGEN COMPONENTS OF THE DOMESTIC HEN

Introduction

It was shown in the previous section that dietary energy level had an effect on the nitrogen components of hen urine. After a careful study of that data and the literature, it was decided to study the effect of dietary protein level upon the excretion of uric acid, ammonia, urea, creatine, and amino acid nitrogen. Folin (1905b) based his laws of composition of human urine upon such a study. After some consideration it was thought best to conduct a depletion type study instead of shifting from a high-protein diet to a non-protein diet as Folin did. It was hoped that plotting of the day to day data of the urinary nitrogen components would indicate the protein level necessary for maintenance and at least would indicate the endogenous nitrogen excretion. It was hypothesized that as long as the nitrogen requirement was met or exceeded, the urinary nitrogen excretion would depend on nitrogen intake but if the maintenance requirement was not met then the hen would mobilize body stores and the slope of the line would change.

Experimental Procedure

The hens used in this experiment were taken from a group of strain cross, S. C. White Leghorns which was set aside for this experiment when they were 18 weeks old. Initially they were brooded on the floor and

MOVED TO THE RANGE AT EIGHT WEEKS OF AGE. AT 18 WEEKS OF AGE THEY WERE SELECTED AT RANDOM FROM THE RANGE AND PLACED IN INDIVIDUAL CAGES.

FIFTEEN OF THESE HENS WERE SURGICALLY MODIFIED TO HAVE EXTERIORIZED RECTA ACCORDING TO THE METHOD OF DIXON (1958). ONLY 10 OF THE 15 HENS LIVED OR WERE USABLE. FOUR OF THESE DIED BEFORE SUITABLE CANNULATION TECHNIQUES WERE DEVISED, AS WAS EXPLAINED IN SECTION ONE OF THIS DISSERTATION. OF THE REMAINING 10, ONLY FIVE WERE USED IN THIS TRIAL. TWO OF THE FIVE WHICH WERE NOT SELECTED BECAUSE THE LOCATION OF THE ANAL OPENING WOULD HAVE MADE COLLECTION DIFFICULT. THE OTHER THREE DID NOT CONSUME SUFFICIENT PURIFIED DIET. WHEN THE BIRDS WERE SELECTED FOR THIS STUDY, THEY HAD BEEN SURGICALLY MODIFIED FOR TWO TO SIX MONTHS. THEY WERE LAYING REGULARLY PRIOR TO THE BEGINNING OF THE TRIAL.

THE BIRDS WERE IN LARGE CAGES DURING THE TRIAL AND THE METHOD OF COLLECTION USED WAS THE ONE DESCRIBED IN THE FIRST SECTION OF THIS DISSERTATION WHICH UTILIZED COLLECTION VESSELS MADE OF POLYETHYLENE BOTTLES. THE UNCONSUMED FEED WAS WEIGHED BACK AND DISCARDED AT MIDNIGHT EACH NIGHT, THE NEXT DAY'S RATION WEIGHED OUT, AND THE LIGHTS WERE TURNED OFF. FECES AND URINE COLLECTIONS FOR A GIVEN DAY WERE COMPLETED AT 9:30 O'CLOCK A.M. OF THE FOLLOWING DAY. THIS WAS DONE IN ORDER FOR THE HENS TO COMPLETELY PASS THE UNDIGESTED MATERIAL FROM THE PREVIOUS DAY'S FEEDING. THE HENS HAD TO EAT THE PURIFIED DIET IN ORDER TO HAVE A FECAL EXCRETION ON A GIVEN DAY AND IT WAS REASONED THAT THEY MUST EAT A PORTION OF THE NEXT DAY'S RATION IN ORDER TO CLEAN THEIR DIGESTIVE TRACTS OF THE PREVIOUS DAY'S RATION.

THE FORMULA OF THE DIET IS SHOWN IN TABLE THREE AND TABLE FOUR. THE NO-PROTEIN BASAL PREMIX ACTUALLY ASSAYED 1.0 PERCENT CRUDE PROTEIN (N X 6.25).

TABLE 3

LOW-PROTEIN BASAL DIET

INGREDIENT	PERCENT
CORN STARCH	50
CERELOSE	27.50
VEGETABLE OIL	5.0
AGAR	2.50
METHYL CELLULOSE	1.25
VITAMIN MIX*	1.25
JONES FOSTER SALT MIXTURE	5.625
OYSTER SHELL	2.5
DI CAL	2.5
(AL) ($Si O_2$)	.62
MICRO MINERAL MIX**	125 GR. OR .13
NA CL	.625
CHROMIC OXIDE	.25

* THE VITAMIN SUPPLEMENT SUPPLIED THE FOLLOWING AMOUNT OF THE VARIOUS FACTORS PER POUND OF RATION.

NAME			
THIAMINE HCL	11.30 GM.	2 METHYL NAPTHAQUINONE	2.27 GM.
RIBOFLAVIN	7.26 GM.	NIACIN	68.18
CA PANTOTHENATE	9.08 GM.	CHOLINE	800.00
VITAMIN B_{12}	0.009GM.	DPPD	1.13
PYRIDOXINE HCL	2.720GM.	VITAMIN A	4000 IU
BIOTIN	00.27 GM.	VITAMIN D_3	750 ICU
FOLIC ACID	1.82 GM.	VITAMIN E (2 TOCOPHEROL)	10 IU
INOSITOL	45.45 GM.		

**SUPPLIED THE FOLLOWING AMOUNT OF TRACE ELEMENTS:

MO	10 PPM.	BR	8.0 PPM.
BO	1.5 PPM.	SE	0.1 PPM.

TABLE 4

RATIONS FED IN TRIAL 3

RATION	NO-PROTEIN BASAL	EXTRACTED EGG	CRUDE PROTEIN	EGG PROTEIN	TOTAL RATION ALLOWANCE
	GMS.	GMS.	PERCENT	PERCENT	GMS.
15	80	19.2	14.5	13.7	99.2
13	80	16.6	13.5	12.7	96.6
11	80	14.1	12.2	11.3	94.1
9	80	11.5	10.5	9.6	91.5
7	80	9.0	8.2	7.3	89.0
6	80	7.7	7.2	6.3	87.7
5	80	6.4	6.7	5.8	86.4
4	80	5.1	5.4	4.5	85.1
3	80	3.8	4.2	3.2	83.8
2	80	2.6	3.2	2.2	82.6
1	80	1.3	2.5	1.5	81.3
0	80	0.0	1.0	0.0	80.0

THE GREATER PORTION OF THIS WAS PROBABLY DUE TO THE VITAMIN SUPPLEMENT ALTHOUGH THE CORN STARCH UNDOUBTEDLY CONTAINED A SMALL AMOUNT OF CRUDE PROTEIN. THE RATIONS WERE CALCULATED TO YIELD A STARTING PROTEIN LEVEL OF 15 PERCENT. THE RATION NUMBER IN THE LEFT-HAND COLUMN OF TABLE FOUR CORRESPONDS TO THE PROTEIN LEVEL CALCULATED FROM THE NITROGEN CONTENT OF THE EXTRACTED EGG AND THE NO-PROTEIN BASAL PRIOR TO MIXING AND PELLETING. THE DECREASE IN PROTEIN LEVEL FROM THE THEORETICAL VALUE TO THAT FOUND BY THE KJELDAHL ANALYSIS IS BELIEVED TO BE DUE TO THE ADDITION OF WATER DURING PELLETING ALTHOUGH THE PELLETS WERE DRIED BEFORE USE.

THE RIGHT HAND COLUMN (TABLE FOUR) GIVES THE ACTUAL FEED ALLOWED WHEN THE BIRDS WERE FED THE FINISHED RATION. THIS WAS CALCULATED TO REPRESENT THE MAXIMUM AMOUNT OF THE NO-PROTEIN BASAL WHICH THE HENS HAD CONSUMED DURING A ONE WEEK PERIOD ONE MONTH PRIOR TO THE BEGINNING OF THE EXPERIMENT. DURING THE TIME THIS VALUE WAS DETERMINED THE HENS RECEIVED A 15 PERCENT PROTEIN RATION FORMULATED FROM THE BASAL NO-PROTEIN DIET, ZIEN, DRACKETT PROTEIN, AND WHOLE DRIED EGG. ZEIN CONTRIBUTED FOUR PERCENT, DRACKETT CONTRIBUTED 10 PERCENT, AND WHOLE EGG CONTRIBUTED ONE PERCENT OF THE PROTEIN IN THIS DIET. IF A HEN DID NOT EAT 25 PERCENT OF THE AVERAGE INTAKE FOR A GIVEN DAY, THE DATA FOR THAT HEN WAS NOT USED IN COMPUTING THE DAILY AVERAGE.

THE EXTRACTED EGG WAS PREPARED IN THE POULTRY DEPARTMENT LABORATORY AT THE LOUISIANA STATE UNIVERSITY. DRIED WHOLE EGG (ANHEUSER BUSCH, ST. LOUIS, MO.) WAS FIRST EXTRACTED FOR 24 HOURS WITH A 3:2 MIXTURE OF ACETONE AND ALCOHOL FOLLOWED BY A 24 HOUR EXTRACTION WITH DIETHYL ETHER. THE PINKISH-WHITE POWDER OBTAINED HAD A PROTEIN CONTENT OF 79 PERCENT (N X 6.25). THE PRODUCT WAS VERY DIGESTIBLE; HEN THREE, ON RATIONS

13, 11, 9 AND 7 DIGESTED 100 PERCENT OF HER PROTEIN INTAKE. THIS SEEMS TO BE UNUSUALLY GOOD BUT THE DIGESTIBILITY COEFFICIENTS WERE ALL UNIFORMLY HIGH AT THE HIGHER LEVELS OF INTAKE.

THE EXPERIMENTAL PLAN WAS TO REDUCE THE PROTEIN INTAKE EACH DAY IN SUCH A MANNER THAT ON THE 12TH DAY AND FOR TWO DAYS THEREAFTER THE HENS WOULD RECEIVE NO SUPPLEMENTAL EGG PROTEIN. ON THE 15TH DAY THE HENS RECEIVED NO FEED WHATEVER. WATER WAS BEFORE THE BIRDS AT ALL TIMES.

THE DAILY URINE COLLECTIONS WERE HANDLED AS DESCRIBED IN THE APPENDIX (SECTION II). THE FECES WERE WEIGHED IN TARED COLLECTION BOTTLES, TRANSFERRED TO SIX OUNCE WIDE MOUTHED, POLYETHYLENE BOTTLES AND FROZEN IMMEDIATELY.

THE TOTAL NITROGEN IN THE FEED, FECES AND URINE WAS DETERMINED BY THE KJELDAHL PROCEDURE. IN ADDITION, THE AMOUNT OF URIC ACID NITROGEN, AMMONIA NITROGEN, UREA NITROGEN, CREATINE NITROGEN, AND CREATININE NITROGEN, WAS DETERMINED FOR EACH INDIVIDUAL URINE COLLECTION. THE AMINO ACID NITROGEN WAS DETERMINED ON A COMPOSITE SAMPLE OF 0.5 PERCENT OF THE TOTAL URINE COLLECTED EACH DAY (ALL HENS). THIS WAS DONE BECAUSE IN TRIAL TWO IT WAS FOUND THAT THE AMINO ACID NITROGEN REPRESENTED ONLY 0.7 TO 1.0 PERCENT OF THE TOTAL URINARY NITROGEN AND THE DETERMINATION WAS NOT WELL ADAPTED TO NUMEROUS, ROUTINE DETERMINATIONS.

TOWARD THE END OF THE TRIAL (12TH DAY) THE AUTHOR INADVERTANTLY TASTED A URINE SAMPLE AND IT TASTED VERY SWEET. IT SEEMED APPROPRIATE, THEREFORE, TO DETERMINE CARBOHYDRATE AND THE ANTHRONE METHOD WAS USED. THE DILUTION AND SAMPLING TECHNIQUES AND THE METHODS OF ANALYSIS FOR ALL THESE URINARY COMPONENTS APPEAR IN THE APPENDIX (SECTION II).

Results and Discussion

The results of this trial appear in tabular form in the Appendix (Section I). The nitrogen balance data are summarized in Table I and the urinary nitrogen data, except the amino acid nitrogen data, are summarized in Table II. Graphic presentations of these data appear in Figures 17 through 25. In these figures, the particular component being considered is plotted on the Y axis against the day of depletion and the corresponding egg protein level in the ration on the X axis.

In Figure 17 the relationship between the supplementary protein level and the absorbed nitrogen is presented. With the exception of one point (third day), the relationship is a linear one. On the third day the feed consumption dropped, and the nitrogen absorption dropped correspondingly. The plateau between the sixth and seventh day was probably due to the small difference in protein level between the two rations.

The effect of protein level on the urinary nitrogen excretion (Figure 18) shows a linear relationship down to a level of 6.3 percent supplementary protein. Beyond that point there are increases in nitrogen excretion preceded and followed by lower nitrogen excretions. However, there is an overall decrease in urinary nitrogen excretion as the protein level of the ration decreases. Note the increase in excretion between the 14th and 15th days. On the latter day the birds received no feed whatever. The observed pattern indicates that the energy intake on the 13th and 14th days probably spared body protein. A comparison of the values for the third day in Figure 17 and Figure 18 leads one to surmise that ration protein level has more effect on the urinary nitrogen excretion than the amount of absorbed nitrogen.

FIGURE 17

DAY OF DEPLETION AND PROTEIN LEVEL VS. ABSORBED NITROGEN

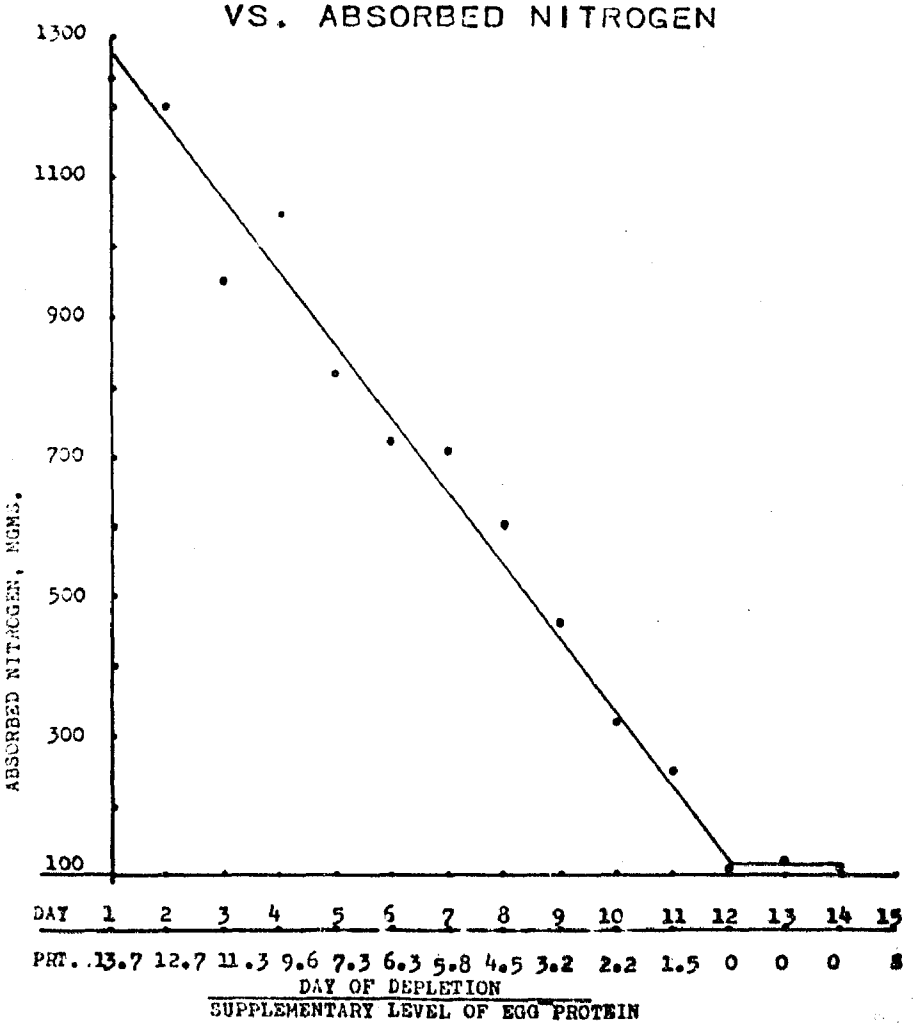
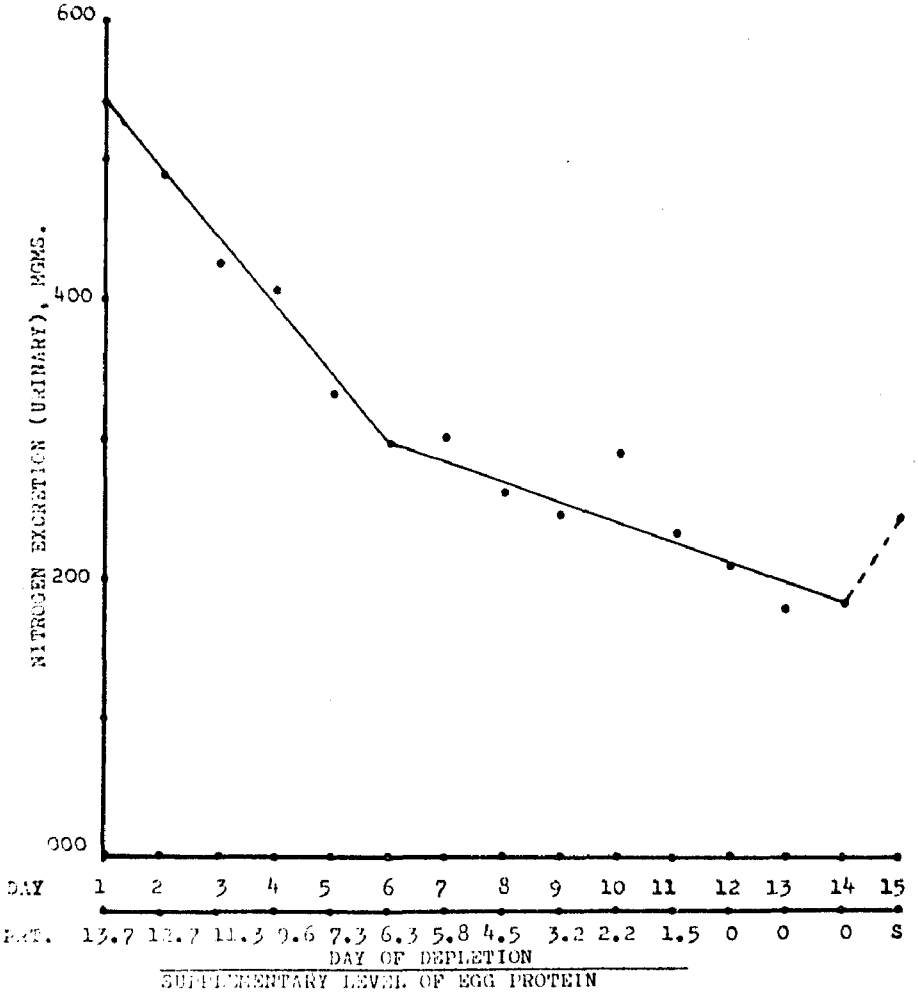


FIGURE 18

DAY OF DEPLETION AND PROTEIN LEVEL VS. URINARY NITROGEN EXCRETION



In Figure 19 the effect on the uric acid excretion is observed. The relationship between protein level and uric acid excretion is nearly linear between the 12.7 percent and the 6.3 percent supplementary protein levels with a plateau between the 6.3 percent and 5.8 percent levels. Beyond the seventh day the excretion is very erratic from day to day but from the first day to the 14th day there is an overall, gradual drop in uric acid excretion.

The ammonia nitrogen excretion (Figure 20) follows the same pattern as the uric acid excretion with the exception that it is much more erratic from day to day. If the source of the urinary ammonia is the oxidative deamination of the circulating amino acids (Lotspeich and Pitts, (1947)), then it would seem likely that such a drop as that occuring between the fifth and sixth days must reflect a change in the level of circulating amino acids. Of ammonia excretion one can say that a decrease in the protein level of the ration will bring about an overall decrease in ammonia excretion.

The urinary amino acid nitrogen, according to the current theory, is a spill-over from the blood which is not resorbed in the kidney tubules. The data from this experiment (Figure 21) does not particularly support this view unless the oxidative deamination of the amino acids in the kidneys is reduced during protein depletion. (Compare Figures 20 and 21). Certainly, the amino acid nitrogen excretion remained very constant during this experiment.

The urea nitrogen excretion was very erratic from day to day (Figure 22). The energy level of the ration did not favor urea excretion particularly but there was an overall decrease in urea excretion from the first to the fourteenth day of the experiment. Again note the break in the overall

FIGURE 19

DAY OF DEPLETION AND PROTEIN LEVEL VS. URIC ACID NITROGEN EXCRETION

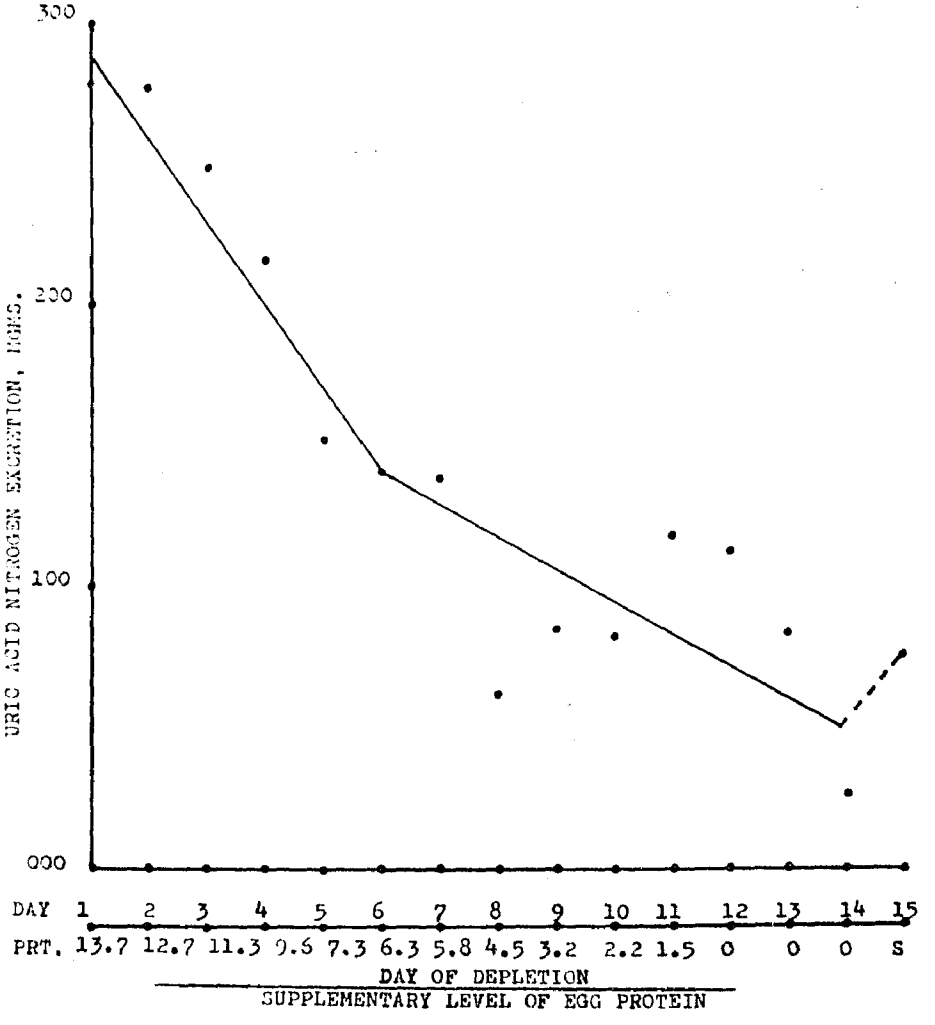


FIGURE 20
DAY OF DEPLETION AND PROTEIN LEVEL VS. AMMONIA NITROGEN EXCRETION

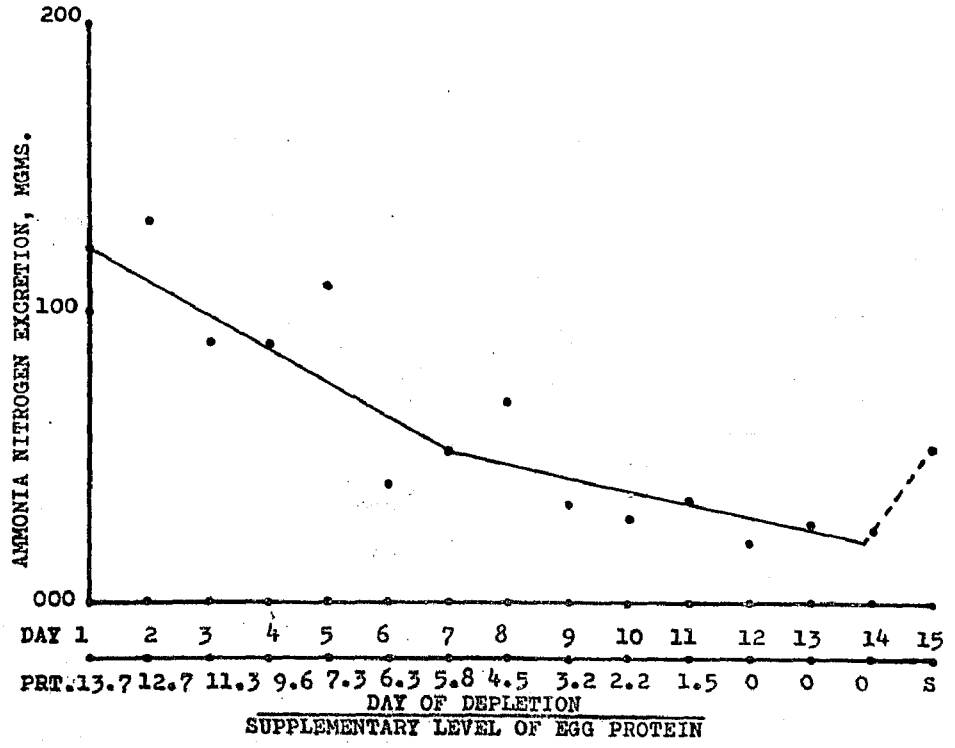


FIGURE 21

DAY OF DEPLETION AND PROTEIN LEVEL VS. AMINO ACID NITROGEN EXCRETION

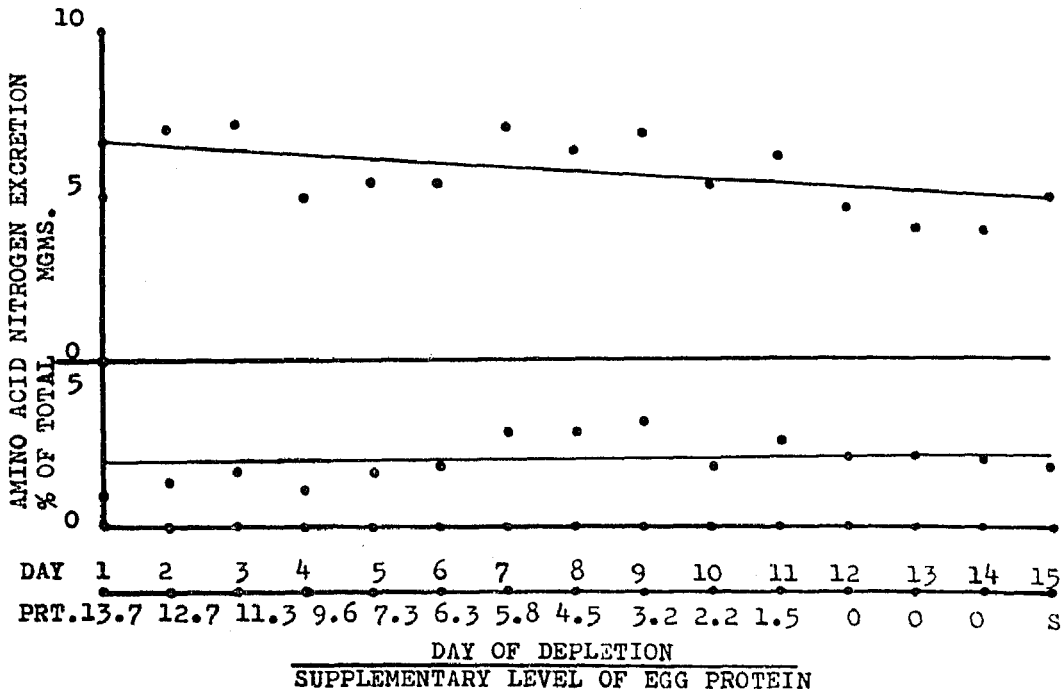
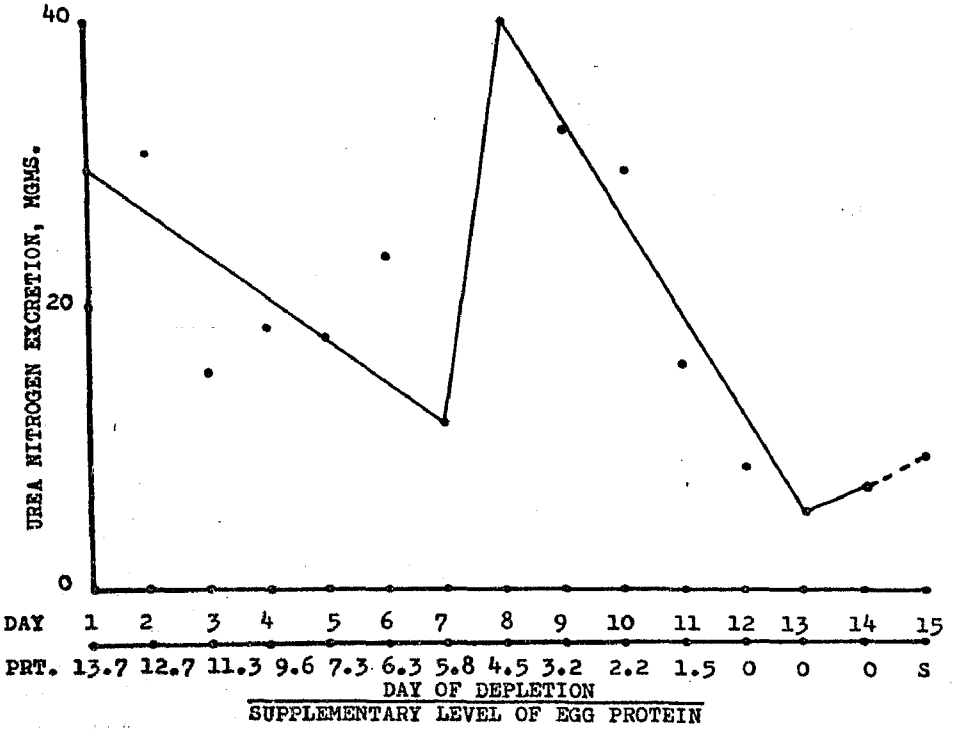


FIGURE 22

DAY OF DEPLETION AND PROTEIN LEVEL VS. UREA NITROGEN EXCRETION



PATTERN WHICH OCCURS AFTER THE SEVENTH DAY. IF THE UREA IS DERIVED SOLELY FROM ARGININE, THERE WAS A CONSIDERABLE MOBILIZATION OF ARGININE ON THE EIGHTH DAY AND INDEED, THE CREATINE EXCRETION (FIGURE 23) SEEMS TO INDICATE SOME INCREASE IN ARGININE CATABOLISM DID OCCUR DURING THIS PERIOD. THE DATA REPRESENTS THE TOTAL EXCRETION OF CREATINE AND CREATININE.

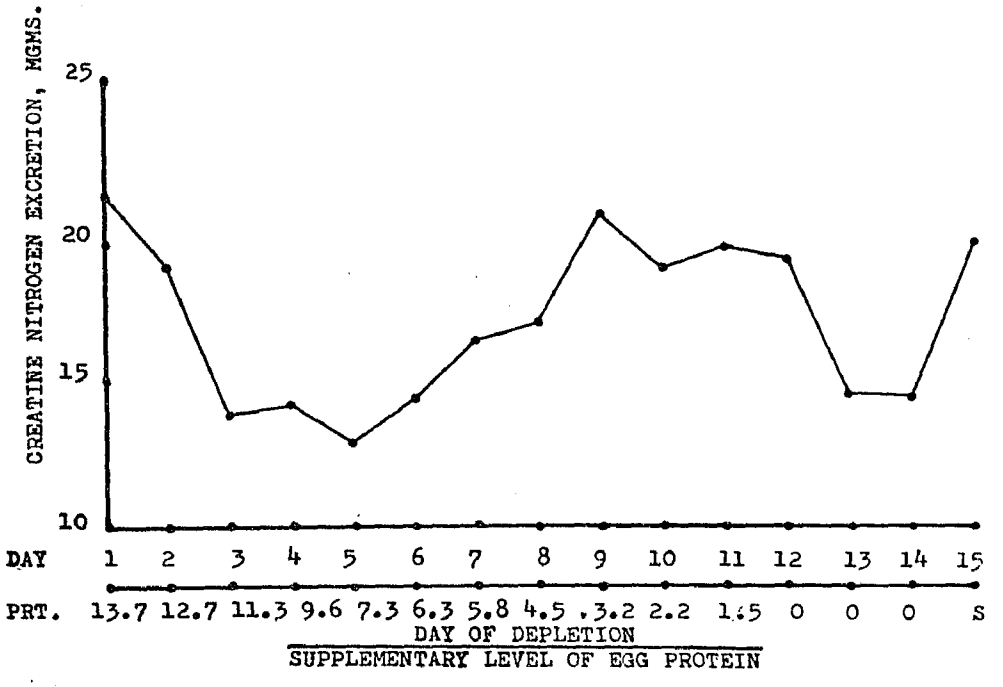
THE FLUCTUATIONS IN CREATINE EXCRETION SEEM TO REFLECT A VERY POWERFUL INATE ABILITY OF THE HEN TO CONTROL HER NITROGEN CATABOLISM WITHIN CERTAIN LIMITS. CERTAINLY, THE VARIATIONS IS NOT VERY GREAT WHEN COMPARED TO THE TOTAL URINARY NITROGEN EXCRETION, BUT, THE LOWEST VALUE REPRESENTS A DECREASE OF APPROXIMATELY 33 PERCENT FROM THE FIRST DAY OF THE EXPERIMENT.

IT IS INTERESTING THAT THE EXCRETION OF THE UNKNOWN NITROGEN COMPONENTS (FIGURE 24) TENDED TO VARY IN A MANNER SIMILAR TO THAT OF CREATINE. IF THE COMPLETE PATTERN IS CONSIDERED THERE IS A TENDENCY FOR THIS EXCRETION TO REMAIN CONSTANT.

THE DATA IN FIGURE 25 WERE UNEXPECTED. IN THE CONCEPTION OF THIS EXPERIMENT IT WAS NOT SUSPECTED THAT THE PROTEIN LEVEL OF THE RATION WOULD AFFECT THE CARBOHYDRATE EXCRETION. THERE IS LITTLE DOUBT THAT THERE WAS A RELATIONSHIP UNDER THE CONDITIONS OF THIS EXPERIMENT. THE RISE IN CARBOHYDRATE EXCRETION BECAME VERY PRONOUNCED BETWEEN THE SEVENTH AND THIRTEENTH DAY OF THE EXPERIMENT AND AT THE SAME TIME THERE WAS A VERY ERRATIC EXCRETION OF THE NITROGEN COMPONENTS CHARACTERIZED BY DAY TO DAY INCREASES AND DECREASES. THIS RELATIONSHIP WAS QUITE EVIDENT IN THE EXCRETION OF UREA, CREATINE AND URIC ACID AND LESS EVIDENT IN THE EXCRETION OF AMMONIA AND THE UNKNOWN COMPONENT(S). THE AMINO NITROGEN COMPONENT SHOWS NO MARKED CHANGE DURING THIS PERIOD.

FIGURE 23

DAY OF DEPLETION AND PROTEIN LEVEL VS. CREATINE NITROGEN EXCRETION[1]



[1] TOTAL EXCRETION OF CREATINE AND CREATININE NITROGEN

FIGURE 24

DAY OF DEPLETION AND PROTEIN LEVEL VS. UNKNOWN NITROGEN EXCRETION

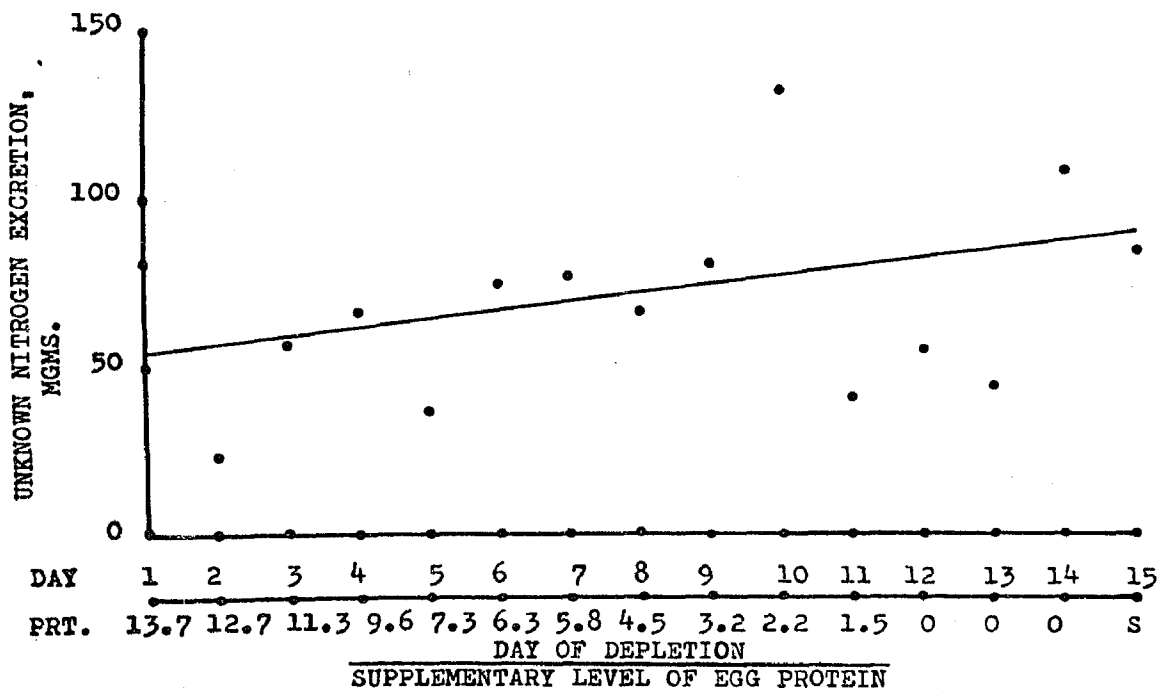
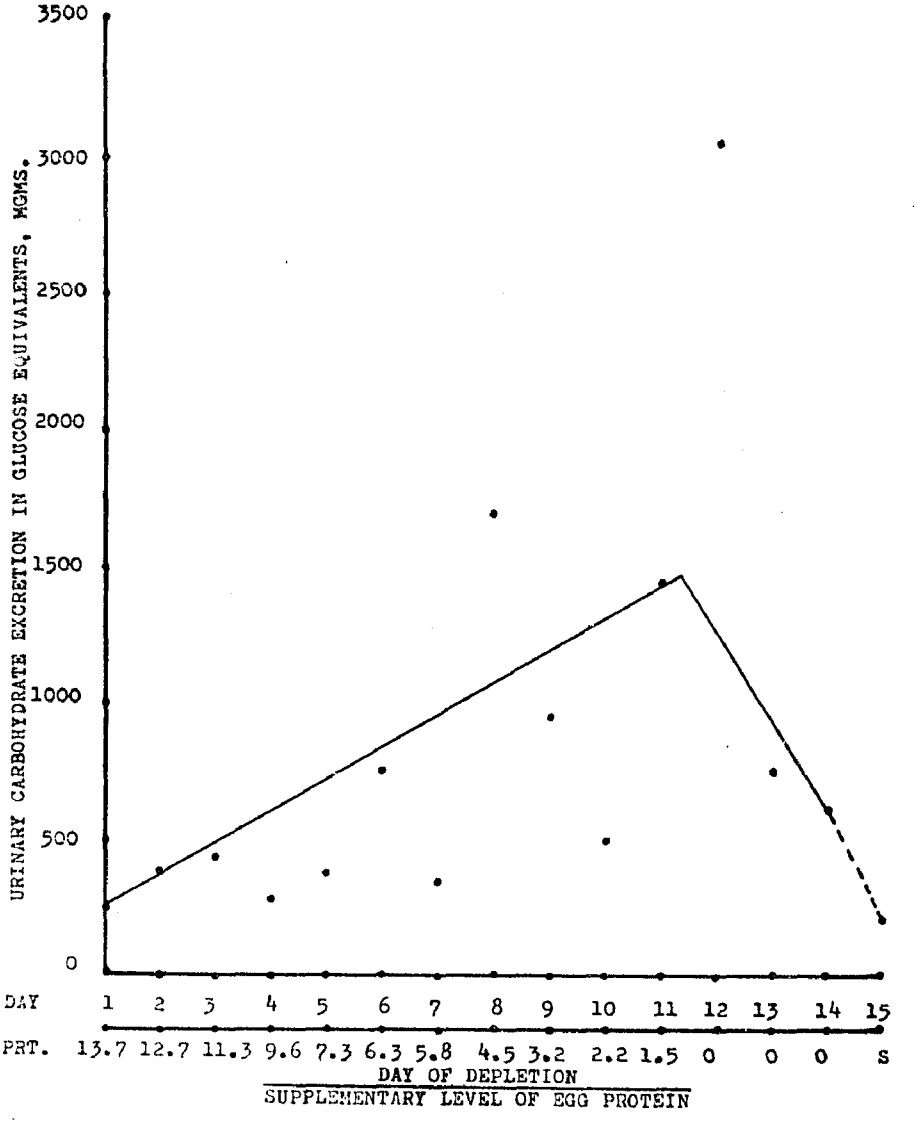


FIGURE 25

DAY OF DEPLETION AND PROTEIN LEVEL VS. URINARY CARBOHYDRATE EXCRETION



THE DATA PRESENTED HERE INDICATE THAT THE HEN HAS AN ABILITY TO ADJUST HER NITROGEN METABOLISM IN ACCORDANCE WITH THE REQUIREMENTS ESTABLISHED BY HER PROTEIN INTAKE. A STUDY OF THE VARIOUS GRAPHS AND TABLES INDICATES THAT THE MAINTENANCE REQUIREMENT FOR THE GROUP WAS MET AT ABOUT 6.3 TO 5.8 PERCENT SUPPLEMENTAL EGG PROTEIN. A STUDY OF THE WEIGHT GAINS (TABLE I, APPENDIX SECTION I) SHOWS THAT PRIOR TO THE SEVENTH DAY THE WEIGHT GAIN OR LOSS OF THE INDIVIDUAL HENS DEPENDED MORE UPON FEED INTAKE THAN UPON RATION PROTEIN LEVEL. AFTER THE SEVENTH DAY THE HENS CONSISTENTLY LOST BODY WEIGHT EXCEPT IN CERTAIN SPECIFIC INSTANCES. THE HEAVIER HENS BEGAN LOSING WEIGHT BEFORE THEIR LIGHTER SISTERS.

TEN OF THE 15 EGGS LAID DURING THE TRIAL WERE LAID ON OR BEFORE THE SEVENTH DAY. THE AVERAGE HEN GAIN ON THE DAY AN EGG WAS LAID WAS -16 GMS. UP THROUGH THE SEVENTH DAY BUT WAS -87 GMS. AFTER THE SEVENTH DAY. A COMPARISON OF THE HEN WEIGHTS BETWEEN THE MORNING OF THE FIRST DAY (10/16) AND THE MORNING OF THE EIGHTH DAY (10/23) SHOWED THAT ONLY THE HEAVIER HEN (No. 3) HAD SUSTAINED A CONSISTENT WEIGHT LOSS. ALTHOUGH THE WEIGHT LOSS WAS NEGLIBIBLE, THE EGG PRODUCTION WAS REDUCED. IN THE WEEK PRIOR TO THE EXPERIMENT THE HENS WERE FED A PURIFIED RATION CONTAINING 15 PERCENT PROTEIN (FOUR PERCENT FROM ZEIN, 10 PERCENT FROM DRACKETT, AND ONE PERCENT FROM WHOLE DRIED EGG). DURING THIS PERIOD THE FIVE HENS LAID 18 EGGS.

THERE WAS NO INTENTION TO RELATE THE LEVEL OF EGG PROTEIN IN THE RATION TO EGG PRODUCTION FROM THE BEGINNING TO THE END OF THE EXPERIMENT BUT THE FACT THAT EGG PRODUCTION AFTER THE SEVENTH DAY WAS ACCOMPANIED BY SUCH A GREAT WEIGHT LOSS DOES INDICATE THAT THE HENS REQUIRED AT LEAST 5.8 PERCENT EGG PROTEIN JUST FOR THE MAINTENANCE OF NORMAL BODY FUNCTIONS. THIS CORRESPONDS TO AN INTAKE OF ABOUT 3.8 GRAMS OF EGG PROTEIN, CALCULATED

FROM THE AVERAGE FEED CONSUMPTION FOR THE SEVENTH DAY OF THE TRIAL.

ARIYOSHI (1957) STATED THAT THE MAINTENANCE PROTEIN LEVEL MAY BE LOWER THAN 1.8 GMS. OF THE WHOLE EGG PROTEIN PER DAY. IN HIS TRIALS HE ACTUALLY DEPLETED HIS BIRDS OVER A PERIOD OF 42 DAYS, TO DETERMINE THE ENDOGENOUS NITROGEN EXCRETION. THEN HE ALLOWED THEM A PERIOD OF 22 DAYS TO REGAIN SOME OF THEIR LOST WEIGHT, AND, AGAIN PLACED THEM ON A DEPLETION RATION FOR 14 DAYS (ONE GM. OF DRIED EGG PER DAY AS A SOURCE OF PROTEIN). HE THEN FED DIFFERENT INDIVIDUAL BIRDS EITHER THREE OR FIVE PERCENT WHOLE EGG FOR NINE DAYS. A CLOSE EXAMINATION OF THE WEIGHT DATA SHOWS THAT SOME OF THE BIRDS BECAME ADAPTED TO THE LOW-PROTEIN RATIONS WHILE OTHERS DID NOT. THIS MAY HAVE AFFECTED HIS DETERMINATION OF THE ENDOGENOUS NITROGEN EXCRETION.

IN THE STUDY REPORTED IN THIS DISSERTATION THERE WERE TWO PLATEAUS IN THE URINARY NITROGEN EXCRETION (FIGURE 18). THE NITROGEN EXCRETION AT THE MAINTENANCE PROTEIN LEVEL (SEVENTH DAY) WAS 304 MGMS. AND ON THE NON-PROTEIN RATION (DAYS 13 AND 14) THE URINARY EXCRETION WAS 180 TO 186 MGMS. THE URINARY NITROGEN EXCRETION AT THE MAINTENANCE LEVEL WAS ABOUT 100 MGMS. HIGHER THAN THAT FOUND IN ARIYOSHI'S TABLES AND HIS DATA REPRESENTED HEAVIER BIRDS. HOWEVER, HE USED COCKS AND CAPONS AND THE SEX DIFFERENCE MAY INFLUENCE THE URINARY NITROGEN EXCRETION AT MAINTENANCE PROTEIN LEVELS. THE URINARY NITROGEN EXCRETION ON THE NO-PROTEIN DIETS FED IN THIS STUDY WAS ABOUT 42 MGMS. LOWER THAN THAT CALCULATED FROM ARIYOSHI'S WORK (185 MGMS. AGAINST 227 MGMS.). HERE AGAIN THE SEX DIFFERENCE AND/OR THE DIFFERENT METHOD OF DEPLETION MAY HAVE INFLUENCED THE RESULT.

IT WAS NOT THE INTENTION IN THE DESIGN OF THIS STUDY TO DETERMINE THE

BIOLOGICAL VALUE OR DIGESTIBILITY OF THE EGG PROTEIN. THE DATA ARE INCLUDED IN THE APPENDIX SECTION I, TABLE I FOR POSSIBLE LATER USE OR SPECULATION AND WILL NOT BE FURTHER DISCUSSED.

THE URINARY NITROGEN EXCRETION DATA ARE CONSIDERED ADEQUATE ENOUGH TO GIVE ONE AN IDEA OF THE EFFECT OF LOWERING THE PROTEIN LEVEL IN A RATION WHILE ALLOWING A POSSIBLE CONSTANT ENERGY INTAKE. OF THE URINARY NITROGEN COMPONENTS STUDIED, ONLY THE UREA AND CREATINE NITROGEN EXCRETIONS DID NOT FOLLOW THE EXPECTED PATTERN. IT WAS EXPECTED THAT THE CREATINE EXCRETION WOULD REMAIN MORE CONSTANT AND THAT THE UREA EXCRETION WOULD DECREASE WITH THE DECREASE IN PROTEIN LEVEL. WHEN THE RESULTS OF THIS EXPERIMENT ARE COMPARED WITH THE RESULTS OF TRIAL TWO, IT IS DIFFICULT TO RELATE ALL OF THE UREA EXCRETION WITH ARGININE METABOLISM. A GREAT DEAL MORE WORK ALONG THIS LINE IS INDICATED.

THE INCREASED URINARY CARBOHYDRATE EXCRETION ASSOCIATED WITH THE DECREASE IN PROTEIN LEVEL GIVES RISE TO ANOTHER INTERESTING QUESTION. FROM THE DATA HEREIN PRESENTED, THIS PHENOMENA CAN ONLY BE ASSOCIATED WITH AN ATTEMPT OF THE BIRDS TO ADAPT TO A LOWERED PROTEIN LEVEL IN THE RATION; THE FUNDAMENTAL RELATIONSHIP IS OBSCURE.

SUMMARY

A 14 DAY PROTEIN DEPLETION STUDY WAS RUN WITH FIVE SURGICALLY MODIFIED S. C. WHITE LEGHORN FEMALES. THE SUPPLEMENTARY EGG PROTEIN LEVEL OF THE RATION WAS DECREASED FROM 13.7 PERCENT ON THE FIRST DAY TO ZERO ON THE 12TH DAY. ON THE 15TH DAY THE HENS WERE STARVED. EACH DAY SEPARATE COLLECTIONS OF URINE AND FECES WERE MADE. THE TOTAL NITROGEN OF THE FEED, FECES, AND URINE WERE DETERMINED, IN ADDITION THE DAILY EXCRETION OF URIC ACID, AMMONIA, UREA, AND CREATINE NITROGEN WERE DETERMINED. AMINO ACID

NITROGEN WAS DETERMINED ON A COMPOSITE SAMPLE OF URINE REPRESENTING 1/200TH OF THE TOTAL URINE EXCRETED.

IT WAS FOUND THAT:

(1) AS THE PROTEIN LEVEL DECREASED THE AMMONIA AND URIC ACID NITROGEN EXCRETION DECREASED.

(2) THE UREA NITROGEN EXCRETION IN THE URINE VARIED ERRATICALLY FROM DAY TO DAY BUT FROM THE BEGINNING TO THE END OF THE EXPERIMENT THERE WAS A CONSIDERABLE DECREASE IN THE EXCRETION OF UREA NITROGEN.

(3) THE CREATINE NITROGEN VARIED A GREAT DEAL THROUGHOUT THE EXPERIMENT, BUT IF THE CREATINE EXCRETION FOR THE WHOLE EXPERIMENT IS CONSIDERED, THERE WAS A TENDENCY FOR THE BIRD TO MAINTAIN A CONSTANT LEVEL OF CREATINE EXCRETION.

(4) THE EXCRETION OF AMINO ACID NITROGEN REMAINED FAIRLY CONSTANT THROUGHOUT THE EXPERIMENT.

(5) THE UNKNOWN NITROGEN EXCRETION WAS QUITE VARIABLE WITH NO SUBSTANTIAL DECREASE OR INCREASE FROM THE BEGINNING TO THE END OF THE EXPERIMENT. THE VARIATION DID NOT SEEM TO BE RELATED TO DIETARY TREATMENT.

(6) AS THE PROTEIN LEVEL DECREASED THERE WAS A MARKED INCREASE IN URINARY CARBOHYDRATE AS DETERMINED BY THE ANTHRONE METHOD.

FROM THE DATA HEREIN PRESENTED THE CONCLUSION WAS DRAWN THAT THE HEN CAN ADAPT VERY READILY TO DECREASES IN RATION PROTEIN LEVEL UNTIL THAT LEVEL DECREASES TO A POINT WHICH REDUCES PROTEIN INTAKE BELOW HER MAINTENANCE NEEDS. BELOW MAINTENANCE THE HEN LOSES WEIGHT AS SHE ATTEMPTS TO ADAPT HERSELF TO THE LOWER PROTEIN LEVEL.

SUMMARY

SURGICAL MODIFICATIONS WERE MADE WHICH PERMITTED THE SEPARATE COLLECTION OF FECES AND URINE FROM CHICKENS. THE TECHNIQUES WERE REFINED AND UTILIZED IN A FUNDAMENTAL STUDY OF NITROGEN METABOLISM.

IN EXPERIMENT ONE IT WAS FOUND NECESSARY TO ADD AN ACID PRESERVATIVE IN THE URINE COLLECTION VESSELS AND BORIC ACID PROVED TO BE AN EXCELLENT PRESERVATIVE IN THESE EXPERIMENTS. THERE WAS NO DIFFERENCE IN THE RESPONSE OF SURGICALLY MODIFIED HENS OR NORMAL HENS TO DIETARY TREATMENT.

BIRDS WITH EXTERIORIZED RECTA WERE FOUND TO GIVE MORE CONSISTENT QUANTITATIVE COLLECTIONS AND WERE SELECTED AS THE BIRD OF CHOICE FOR NUTRITION EXPERIMENTS. IT WAS FOUND NECESSARY TO FIT THESE BIRDS WITH GLASS CANNULAE AND TO FEED THEM A HIGHLY DIGESTIBLE DIET IN ORDER TO MAINTAIN FECES EXCRETION.

THE SURGICALLY MODIFIED HENS WERE USED IN TWO EXPERIMENTS WHICH WERE DESIGNED TO INDICATE CERTAIN FUNDAMENTAL ASPECTS OF NITROGEN METABOLISM. EXPERIMENT TWO INVOLVED ISO-PROTEIN RATIONS IN WHICH THE ENERGY LEVEL WAS VARIED BY SUBSTITUTING ANIMAL TALLOW FOR RICE HULLS. THE PROTEIN LEVEL WAS 15 PERCENT AND THE ENERGY LEVELS WERE 900 AND 800 CALORIES OF PRODUCTIVE ENERGY PER POUND OR 1310 AND 1086 CALORIES OF METABOLIZABLE ENERGY PER POUND, RESPECTIVELY. EXPERIMENT THREE WAS DESIGNED SO THAT EACH HEN WAS ALLOWED 80 GMS. OF A NO-PROTEIN DIET PER DAY. FOR THE FIRST 11 DAYS THIS DIET WAS SUPPLEMENTED WITH DAILY, DECREASING INCREMENTS OF EXTRACTED WHOLE EGG. THE TWO EXPERIMENTS HAD A COMMON VARIABLE, A CHANGING "ENERGY: PROTEIN"

RATIO (THE CALORIES OF METABOLIZABLE ENERGY FROM CARBOHYDRATE AND FAT PER UNIT WEIGHT DIVIDED BY THE PERCENT OF PROTEIN PER UNIT WEIGHT). SINCE THE INGREDIENTS SUPPLYING THE PROTEIN AND ENERGY WERE DIFFERENT IN THE TWO EXPERIMENTS, ONLY THEORETICAL COMPARISONS CAN BE MADE.

IN EXPERIMENT TWO THE METABOLIZABLE ENERGY WAS DECREASED FROM ABOUT 1310 CALORIES PER POUND TO ABOUT 1086 CALORIES PER POUND BY DECREASING THE AMOUNT OF FAT IN THE RATION AND HOLDING THE PROTEIN CONSTANT. THIS DECREASED THE ENERGY: PROTEIN RATIO FROM 85.4 TO 70.5.

THIS DECREASE IN THE ENERGY: PROTEIN RATIO BROUGHT ABOUT A DECREASE IN THE AMOUNT OF AMMONIA NITROGEN AND AMINO ACID NITROGEN EXCRETED AND AN INCREASE IN THE AMOUNT OF URIC ACID NITROGEN AND UREA NITROGEN EXCRETED. THE TOTAL CREATINE AND CREATININE NITROGEN REMAINED CONSTANT DURING THE TWO COLLECTION PERIODS.

IN EXPERIMENT THREE THE ENERGY: PROTEIN RATIO WAS WIDENED BY ALLOWING A PREDETERMINED MAXIMUM INTAKE OF NUTRIENTS OTHER THAN PROTEIN (A TOTAL OF 80 GMS. DAILY) AND REDUCING THE LEVEL OF SUPPLEMENTAL, EGG PROTEIN EACH DAY FOR 12 DAYS. THE METABOLIZABLE ENERGY FROM CARBOHYDRATES AND FAT OF THESE RATIONS WAS QUITE HIGH, 1380 CALORIES PER POUND OF 14.7 PERCENT PROTEIN DIET AND ABOUT 1540 CALORIES PER POUND OF THE NO-PROTEIN DIET. THIS GIVES AN ENERGY: PROTEIN RATIO INCREASE FROM 100.7 ON THE FIRST DAY OF THE EXPERIMENT TO 1540 ON THE LAST DAY OF THE EXPERIMENT. (THIS TAKES ONLY THE CRUDE PROTEIN OF THE SUPPLEMENTAL, EXTRACTED, EGG POWDER INTO CONSIDERATION).

IF ONE COMPARES THE URINARY NITROGEN DATA BETWEEN EXPERIMENT TWO AND EXPERIMENT THREE HE FINDS THAT URIC ACID CONTRIBUTED ABOUT 74 PERCENT OF THE URINARY NITROGEN WHEN A DIET WITH AN ENERGY: PROTEIN RATIO OF 85.4

WAS FED AND CONTRIBUTED ABOUT 49 PERCENT OF THE URINARY NITROGEN WHEN A DIET WITH AN ENERGY: PROTEIN RATIO OF 100.7 WAS FED. OF COURSE, IT IS IMPOSSIBLE TO DELINEATE THIS PARTICULAR EFFECT BECAUSE OF THE VARIED SOURCES OF NUTRIENTS BUT SUCH AN EFFECT UPON THE NITROGEN METABOLISM IS CLEARLY INDICATED THROUGHOUT THESE STUDIES. ONE MAY SAY, THEREFORE, THAT WHEN A HEN RECEIVES ENOUGH PROTEIN TO FULFILL OR EXCEED HER MAINTENANCE REQUIREMENTS AN INCREASE IN THE ENERGY: PROTEIN RATIO BRINGS ABOUT A REDUCTION IN THE PERCENT OF URIC ACID IN THE URINE, AN INCREASE IN THE PERCENT OF AMMONIA IN THE URINE, AND A DECREASE IN THE AMOUNT OF URINARY NITROGEN EXCRETION.

IN EXPERIMENT THREE THE HENS HAD ACCESS TO A PREDETERMINED AMOUNT OF FEED BUT THEY SELDOM ATE ALL OF THEIR ALLOWANCE. IT WOULD SEEM THEREFORE THAT FEED INTAKE WAS DEPENDENT ON THE ENERGY LEVEL. AS THE PROTEIN LEVEL DECREASED BELOW 5.8 PERCENT THE DAILY FEED INTAKE TENDED TO INCREASE SO THAT ON THE NO-PROTEIN DIET THE INTAKE WAS NEAR MAXIMUM. IT WOULD SEEM THAT THE HENS ATE ONLY ENOUGH FEED TO SUPPLY THEIR ENERGY NEEDS WHEN THE PROTEIN LEVEL WAS SUCH AS TO ALLOW THEM TO EASILY MEET THE PROTEIN REQUIREMENT FOR MAINTENANCE BUT AS THE PROTEIN LEVEL DECREASED TO SUCH A LEVEL THAT THE MAINTENANCE PROTEIN REQUIREMENT WAS DIFFICULT TO MEET THERE WAS A TENDENCY FOR GREATER INTAKE.

THE INCREASED FEED INTAKE WAS ACCOMPANIED BY AN INCREASE IN THE AMOUNT OF CARBOHYDRATE EXCRETED (AS DETERMINED BY A QUANTATIVE ANTHRONE PROCEDURE). IF THE CARBOHYDRATE EXCRETION HAD GIVEN A LINEAR OR CURVALINEAR PLOT AGAINST RATION PROTEIN LEVEL, ONE COULD SAY THAT THE INCREASED CARBOHYDRATE EXCRETION WAS DUE TO A RELATIVELY SMALL INCREASED INTAKE OF CARBOHYDRATE BUT IT WAS CHARACTERIZED BY A SERIES OF PEAKS AND DIPS. THIS SEEMS TO INDICATE A

RELATIONSHIP BETWEEN ABSOLUTE PROTEIN LEVEL IN THE RATION AND THE UTILIZATION OF CARBOHYDRATE. WITHOUT FURTHER STUDY ONE CAN ONLY SAY THAT AS THE PROTEIN LEVEL OF THE RATION DECREASED THERE WAS AN INCREASE IN CARBOHYDRATE EXCRETION. THIS INDICATED THAT PROTEIN IS IMPORTANT IN THE PROPER UTILIZATION OF CARBOHYDRATE AND/OR THE HENS WERE UNDER CONSIDERABLE STRESS AND WERE TRYING TO ADJUST THEIR METABOLISM.

FROM THE DATA OF THE TWO EXPERIMENTS ONE MAY SAY THAT AN INCREASE IN THE ENERGY: PROTEIN RATIO OF THE DIET BRINGS ABOUT A DECREASE IN UREA EXCRETION. IT WOULD SEEM AS IF THE UREA EXCRETION BELOW THE MAINTENANCE PROTEIN LEVEL REFLECTS THE MOBILIZATION OF TISSUE PROTEINS AS IT IS VERY ERRATIC FROM DAY TO DAY.

THE AMINO ACID NITROGEN EXCRETION TENDS TO REMAIN CONSTANT AND THE CREATINE EXCRETION TENDS TO BE RELATIVELY CONSTANT EXCEPT FOR PERIODS WHEN THE HEN IS TRYING TO ADJUST TO A LOWER NITROGEN INTAKE. THE UNKNOWN URINARY NITROGEN COMPONENTS DECREASED, (BOTH IN AMOUNT OF NITROGEN AND AS A PERCENT OF THE URINARY NITROGEN) AS THE ENERGY WAS INCREASED IN EXPERIMENT TWO. IN EXPERIMENT THREE THE AMOUNT OF UNKNOWN COMPONENTS REMAINED RELATIVELY CONSTANT THROUGHOUT THE EXPERIMENT BUT AS A PERCENT OF THE URINARY NITROGEN IT (THEY) INCREASED WITH A DECREASE IN DIETARY PROTEIN LEVEL.

THE AVERAGE NITROGEN REQUIREMENT FOR MAINTENANCE IN EXPERIMENT THREE WAS APPROXIMATELY 800 MGMS.; THIS INCLUDED 690 MGMS. OF NITROGEN FROM EXTRACTED WHOLE EGG. THE URINARY NITROGEN EXCRETION AT THE MAINTENANCE PROTEIN LEVEL WAS 308 MGMS. AND ON THE NO-PROTEIN RATION THE MINIMUM URINARY NITROGEN EXCRETION WAS ABOUT 180 MGMS.

A STUDY OF THE DATA OF BOTH EXPERIMENTS WOULD LEAD ONE TO ADVANCE TWO HYPOTHESES OF NITROGEN METABOLISM, NAMELY:

(1) IT IS NOT NECESSARILY THE AMOUNT OF PROTEIN IN A RATION BUT THE RELATIONSHIP OF THE PROTEIN TO OTHER NUTRIENTS WHICH DETERMINES THE CHARACTERISTICS OF THE URINARY NITROGEN EXCRETION.

(2) IN ORDER TO CALL A URINE "NORMAL" ONE MUST RIGIDLY DEFINE A "NORMAL" DIET. IN THESE RESPECTS, NO EVIDENCE WAS FOUND WHICH WOULD REFUTE THE CONCLUSIONS OF FOLIN (1905A, 1905B, 1905C) EITHER HIS LAWS FOR THE COMPOSITION OF URINE OR HIS THEORY OF ENDOGENOUS AND EXOGENOUS NITROGEN METABOLISM.

APPENDIX SECTION I

TABULAR DATA FROM EXPERIMENT THREE

TABLE I - NITROGEN BALANCE DATA, EXPERIMENT THREE

TABLE II - THE NITROGEN COMPOSITION OF URINE, EXPERIMENT THREE

TABLE I

Date	Hen No.	Hen Weight	Hen Gain	Nitrogen Intake	Fecal Nitrogen	Absorbed Nitrogen	Percent Digestibility	Urinary Nitrogen	Absorbed Nitrogen Retained	
		Gms.	Gms.	Mgms.	Mgms.	Mgms.	%	Mgms.	Mgms.	Percent
10/16	1	1584	+61	1067.5	160.2	999.2	94	616.3	382.9	38
10/16	2	1873	-32	1373.8	332.8	1134.1	83	600.6	533.5	47
10/16	3	2046	-50	1373.8	145.7	1318.9	96	369.0	949.9	72
10/16	4	1720	+21	1624.4	206.3	1514.5	93	575.1	939.4	62
10/16	5*	1511	-66	----	---	----	--	453.6	----	--
10/16	Average	1805.8	-5.2	1359.9	211.2	1240.0	91	540.2	+699.8	56
10/17	1	1645	-38	895.1	142.1	844.9	94	466.2	378.7	45
10/17	2	1841	-33	1671.6	291.6	1473.1	88	537.3	935.8	64
10/17	3	1996	-51	1242.4	81.2	1252.0	100	453.6	798.4	64
10/17	4	1741	-01	1294.1	150.8	1239.7	96	499.0	740.7	60
10/17	5*	1445	-63	273.9	138.5	219.6	80	421.4	-201.8	--
10/17	Average	1805.8	-30.8	1275.8	166.4	1200.7	95	489.0	+711.7	59
10/18	1	1607	-52	586.5	133.8	544.6	93	319.2	225.4	42
10/18	2	1874	+41	1798.6	243.6	1648.1	92	562.8	1085.3	66
10/18	3	1945	+48	914.9	93.4	912.3	100	386.4	525.9	58
10/18	4	1742	+16	1173.0	187.7	1081.7	92	453.6	628.1	58
10/18	5	1382	+37	684.2	181.7	586.7	86	409.1	177.6	30
10/18		1710.0	+18.0	1031.4	168.0	954.7	93	426.2	+528.5	55

* Not included in the averages

(TABLE I CONTINUED)

Date	Hen No.	Hen Weight	Hen Gain	Nitrogen Intake	Fecal Nitrogen	Absorbed Nitrogen	Percent Digestibility	Urinary Nitrogen	Absorbed Nitrogen Retained	
		Gms.	Gms.	Mgms.	Mgms.	Mgms.		Mgms.	Mgms.	Percent
10/19	1	1555	+73	1074.2	196.9	929.2	90	432.0	537.2	55
10/19	2	1915	-18	1434.5	224.3	1033.3	91	509.6	793.7	61
10/19	3	1993	-23	1321.9	96.5	1316.2	100	388.1	928.1	70
10/19	4	1958	+02	1035.6	122.1	1009.9	98	379.4	630.5	62
10/19	5	1419	+41	796.0	217.2	663.0	83	338.8	324.2	49
10/19	Average	1768.0	+15.0	1132.4	171.4	1052.3	93	409.6	+642.7	61
10/20	1	1628	+09	943.7	241.3	794.3	84	356.2	438.1	55
10/20	2	1897	-26	752.6	110.1	735.6	98	324.8	410.8	56
10/20	3	1970	-19	1010.5	57.9	1043.4	103	313.0	730.4	70
10/20	4	1760	000	1006.5	180.6	922.3	92	396.2	526.1	57
10/20	5	1460	-01	683.2	130.1	637.3	93	274.4	362.9	57
10/20	Average	1743	-7.4	879.3	144.0	826.6	94	332.9	+493.7	60
10/21	1	1637	-67	957.8	116.6	933.1	97	266.7	666.4	71
10/21	2	1871	-13	503.1	244.3	351.9	70	369.9	-18.0	
10/21	3	1951	-39	950.9	200.7	841.0	88	292.6	548.4	65
10/21	4	1760	-33	870.1	214.0	752.5	86	299.6	452.9	60
10/21	5	1459	+06	737.4	148.5	673.1	91	268.5	404.6	60
10/21	Average	1736.6	-29.2	821.9	184.8	728.4	89	299.5	+ 428.9	59

(TABLE I CONTINUED)

Date	Hen No.	Hen Weight	Hen Gain	Nitrogen Intake	Fecal Nitrogen	Absorbed Nitrogen	Percent Digestibility	Urinary Nitrogen	Absorbed Nitrogen Retained	
		Gms.	Gms.	Mgms.	Mgms.	Mgms.	[illegible]	Mgms.	Mgms.	Percent
10/22	1	1570	+52	862.3	102.7	851.5	99	208.2	643.3	76
10/22	2	1858	-10	576.3	226.9	442.5	77	318.9	123.6	28
10/22	3	1912	+73	883.8	171.8	802.8	91	454.7	348.1	43
10/22	4	1727	-24	733.3	235.6	594.1	81	300.4	293.7	49
10/22	5	1465	+09	924.7	130.7	878.2	95	235.2	643.0	73
10/22	Average	1706.4	+20.0	796.0	173.5	713.8	90	303.5	+410.3	57
10/23	1	1622	00	733.6	221.4	604.1	82	289.8	314.3	52
10/23	2*	1848	-115	144.1	64.6	172.6	120	284.5	-111.9	
10/23	3	1985	-23	688.7	125.4	654.1	95	335.3	318.8	49
10/23	4	1703	-14	736.1	253.5	579.0	79	320.2	258.8	45
10/23	5	1474	+04	721.5	216.8	588.9	82	102.5	486.4	83
10/23	Average	1696	-8.2	720.0	204.3	607.0	84	262.0	+ 345.0	57
10/24	1	1622	-65	513.3	147.9	457.3	89	207.5	249.8	54
10/24	2	1733	+53	442.2	96.2	439.1	99	374.6	64.5	15
10/24	3	1962	-72	607.4	107.5	590.7	97	244.0	346.7	59
10/24	4	1689	-85	323.0	138.5	280.9	87	313.2	-32.3	
10/24	5	1478	+40	549.2	27.5	605.9	110	153.4	452.5	75
10/24	Average	1696.8	-25.8	487.0	103.5	474.8	97	258.5	+216.3	46

* Not included in the averages

(TABLE I CONTINUED)

Date	Hen No.	Hen Weight	Hen Gain	Nitrogen Intake	Fecal Nitrogen	Absorbed Nitrogen	Percent Digestibility	Urinary Nitrogen	Absorbed Nitrogen Retained	
		Gms.	Gms.	Mgms.	Mgms.	Mgms.	[illegible].	Mgms.	Mgms.	Percent
10/25	1	1557	-09	273.0	119.2	245.7	90	168.8	76.9	31
10/25	2	1786	+01	409.5	76.3	426.3	104	226.8	199.5	47
10/25	3	1890	+14	399.4	111.2	379.0	95	231.6	147.4	39
10/25	4	1604	-12	401.4	75.6	422.2	105	544.6	-122.4	--
10/25	5	1518	-34	219.9	173.9	130.2	59	282.8	-152.6	---
10/25	Average	1671.0	-8.0	340.6	111.2	320.7	94	290.9	+29.8	9
10/26	1	1548	+34	277.5	86.6	277.5	100 **	243.3	34.2	12
10/26	2	1787	+05	308.0	77.9	308.0	100	218.4	89.6	29
10/26	3	1905	+32	310.8	115.2	310.8	100	236.5	74.3	24
10/26	4	1592	-34	192.2	105.3	192.2	100	285.2	-93.0	--
10/26	5	1484	-63	195.4	79.2	195.4	100	195.3	.1	0.1
10/26	Average	1663.2	-5.2	256.8	92.8	256.8	100	235.7	+21.1	8
10/27	1	1582	-40	129.2	103.4	129.2	100 **	211.7	-82.5	--
10/27	2	1792	-40	127.4	136.8	127.4	100	173.6	-46.2	--
10/27	3	1936	+16	128.5	125.6	128.5	100	225.2	-96.7	--
10/27	4	1558	+09	80.8	64.2	80.8	100	323.3	-242.5	--
10/27	5	1421	+15	88.8	118.7	88.8	100	133.7	-48.9	--
10/27	Average	1657.8	-8.0	110.9	109.7	110.9	100	213.5	-103.4	--

** Digestibility figures for these days are assumed rather than calculated.

(TABLE I CONTINUED)

Date	Hen No.	Hen Weight	Hen Gain	Nitrogen Intake	Fecal Nitrogen	Absorbed Nitrogen	Percent Digestibility	Urinary Nitrogen	Absorbed Nitrogen Retained	
		Gms.	Gms.	Mgms.	Mgms.	Mgms.	%	Mgms.	Mgms.	Percent
10/28	1	1542	-14	120.1	75.7	120.1	100 **	164.6	-44.5	--
10/28	2	1725	+21	127.4	48.7	127.4	100	220.8	-100.6	--
10/28	3	1952	-39	126.2	74.3	126.2	100	226.1	-99.9	--
10/28	4	1567	+05	149.5	92.4	149.5	100	179.1	-29.6	--
10/28	5	1436	+18	106.5	62.2	106.5	100	109.2	-2.7	--
10/28	Average	1649.8	-1.8	125.9	70.6	125.9	100	180.0	-55.5	--
10/29	1	1528	-31	115.1	102.0	115.1	100 **	168.3	-53.2	--
10/29	2	1773	-61	131.5	109.1	131.5	100	185.7	-84.2	--
10/29	3	1913	-94	108.4	48.1	108.4	100	190.1	-81.7	--
10/29	4	1572	-16	128.5	123.9	128.5	100	172.9	-44.4	--
10/29	5	1454	-18	102.1	76.9	102.1	100	210.9	-108.8	--
10/29	Average	1648.0	-44.0	117.1	92.0	117.1	100	185.6	-74.5	--
10/30	1	1417	-66	0	--	---	--	209.2	--	--
10/30	2	1712	-70	0	--	---	--	232.8	--	--
10/30	3	1819	-82	0	--	---	--	285.6	--	--
10/30	4	1556	-77	0	--	---	--	189.1	--	--
10/30	5	1436	-100	0	--	---	--	318.2	--	--
10/30	Average	1588.0	-79.0	0	--	---	--	246.8	--	--

** Digestibility figures for these days are assumed rather than calculated.

TABLE 11

Date	Hen No.	Urinary Nitrogen Excretion in Mgms. Total	Uric Acid	Ammonia	Urea	Creatine[1]	Percentage Composition of Urinary Nitrogen Uric Acid	Ammonia	Urea	Creatine[1]	Unknown	Mgms. Anthrose[2] Glucose
10/16	1	616.3	245.3	172.4	41.4	22.9	39.8	27.9	6.7	3.7	22	333
10/16	2	600.6	400.0	71.5	23.5	18.9	66.6	11.9	3.9	3.1	14	333
10/16	3	369.0	91.4	217.6	17.0	25.4	24.7	58.9	4.6	6.8	5	233
10/16	4	575.1	378.6	28.4	37.0	17.2	65.8	4.9	6.4	2.9	20	166
10/16	5*	453.6	134.0	175.4	32.2	26.9	29.5	38.6	7.0	5.9	19	166
Average		540.2	278.8	122.5	29.7	21.1	49.2	25.9	5.4	4.1	18	246.2
10/17	1	466.2	184.8	239.5	22.7	22.7	39.6	51.3	4.9	4.8	0	279
10/17	2	537.3	419.1	87.0	26.0	13.8	78.0	16.1	4.8	2.5	0	473
10/17	3	453.6	177.9	197.6	33.2	26.8	39.2	43.5	7.3	5.9	4	407
10/17	4	499.0	327.1	3.0	41.6	12.0	65.5	.6	8.3	2.4	23	330
10/17	5*	421.4	266.6	113.8	0.0	19.4	63.2	27.0	0.0	4.6	5	00
Average		489.0	277.2	131.8	30.9	18.8	55.6	27.9	6.3	3.9	7	372
10/18	1	319.2	117.7	157.0	.6	20.7	36.8	49.1	.2	6.4	20	847
10/18	2	562.8	407.2	86.0	1.4	13.7	72.3	15.2	.2	2.4	10	308
10/18	3	386.4	202.8	63.8	10.8	12.5	52.4	16.5	5.3	3.2	22	132
10/18	4	453.6	278.4	11.6	47.6	10.0	61.3	2.5	10.4	2.2	23	902
10/18	5	409.1	242.3	130.0	16.2	11.5	59.2	31.7	3.9	2.8	2	00
Average		426.2	249.7	89.7	15.3	13.7	56.4	23.0	3.9	3.4	15	438

* Not included in averages

2 Total color compared to glucose standards

1 The value for creatine is the combined value of creatine and creatinine.

(TABLE II CONTINUED)

Date	Hen No.	Urinary Nitrogen Excretion in Mgms. Total	Uric Acid	Ammonia	Urea	Creatine[1]	Percentage Composition of Urinary Nitrogen Uric Acid	Ammonia	Urea	Creatine[1]	Un-known	Mgms. Anthrone[2] Glucose
10/19	1	432.0	243.2	177.6	9.4	19.4	56.2	41.1	2.1	4.4	0	165
10/19	2	509.6	206.2	132.4	26.8	17.2	40.4	25.9	5.2	3.3	25	418
10/19	3	388.1	205.3	47.6	26.2	14.7	52.9	12.2	6.8	3.7	24	275
10/19	4	379.4	218.2	28.4	10.0	10.1	57.5	7.4	2.6	2.6	30	253
10/19	5	338.8	213.9	57.6	19.4	9.3	63.1	17.0	5.7	2.7	11	253
Average		409.6	217.4	88.7	18.4	14.1	54.0	20.7	4.4	3.3	18	273
10/20	1	356.2	102.2	143.8	21.6	14.3	28.6	40.3	6.0	4.0	21	473
10/20	2	324.8	244.0	57.0	4.6	11.5	75.1	17.5	1.4	3.5	4	308
10/20	3	313.0	49.0	179.2	5.4	19.5	15.6	57.2	1.7	6.2	19	132
10/20	4	396.2	168.4	127.0	46.0	14.4	42.5	32.0	11.6	3.6	10	341
10/20	5	274.4	210.1	34.6	11.6	5.0	73.2	12.6	4.2	1.8	8	660
Average		332.9	152.9	108.3	17.8	12.9	47.0	31.9	4.9	3.8	12	383
10/21	1	266.7	70.9	74.5	9.6	15.6	26.5	27.9	3.5	5.8	36	1169
10/21	2	369.9	220.8	20.8	73.0	16.3	59.6	5.6	19.7	4.4	11	352
10/21	3	292.6	145.2	5.0	7.3	15.7	49.6	2.8	2.4	5.8	41	594
10/21	4	299.6	105.7	100.0	9.6	16.3	35.2	33.3	3.2	5.4	23	627
10/21	5	268.5	160.7	6.2	17.6	8.2	59.8	2.3	6.5	3.1	28	1035
Average		299.5	140.6	41.3	23.4	14.4	46.1	14.4	7.1	4.9	28	755

1 The value for creatine is the combined value of creatine and creatinine.
2 Total color compared to glucose standards

(TABLE II CONTINUED)

Date	Hen No.	Urinary Nitrogen Excretion in Mgms. Total	Uric Acid	Ammonia	Urea	Creatine [1]	Percentage Composition of Urinary Nitrogen Uric Acid	Ammonia	Urea	Creatine [1]	Unknown	Mgms. Anthrone [2] Glucose
10/22	1	208.2	96.7	21.6	20.3	15.2	46.4	10.4	9.8	7.3	26	385
10/22	2	318.9	172.7	141.6	0.0	29.4	54.2	44.4	0.0	9.2	0	132
10/22	3	454.7	220.8	7.7	14.2	14.0	48.6	1.7	3.1	3.1	43	275
10/22	4	300.4	92.8	76.9	22.7	20.4	30.9	25.6	7.6	6.8	29	550
10/22	5	235.2	108.3	13.1	1.1	7.4	46.0	5.6	.5	3.1	45	385
Average		303.5	138.3	52.2	11.7	17.3	45.2	17.5	4.2	5.9	29	345
10/23	1	289.8	68.7	82.7	30.2	17.9	23.7	29.5	10.4	6.2	31	3894
10/23	2*	284.5	122.9	50.3	69.0	26.7	43.2	17.7	24.3	9.3	5	165
10/23	3	335.3	65.5	119.2	57.7	22.8	19.5	35.6	17.2	6.8	21	646
10/23	4	320.2	103.1	70.2	57.3	22.0	32.2	21.9	17.9	6.9	21	1514
10/23	5	102.5	19.8	0.0	13.8	9.2	19.3	0.0	13.5	8.9	58	7748
Average		262.0	64.3	68.0	39.8	17.9	23.6	26.2	14.8	7.2	27	1700
10/24	1	207.5	28.4	57.7	33.1	21.7	13.7	27.8	15.9	10.5	32	2685
10/24	2	374.6	210.5	12.0	34.9	20.7	56.2	3.4	9.3	5.5	26	264
10/24	3	244.0	51.6	62.5	39.1	25.5	21.1	25.6	16.0	10.5	27	990
10/24	4	313.2	127.8	37.7	35.0	25.2	40.8	12.0	11.2	8.0	28	314
10/24	5	153.4	11.2	0.0	18.9	9.2	7.3	0.0	12.3	6.0	75	517
Average		258.5	85.9	33.9	32.2	20.5	27.8	13.8	12.9	8.1	37	954

* Not included in averages

2 Total color compared to glucose standards

1 The value for creatine is the combined value of creatine and creatinine.

(TABLE II CONTINUED)

Date	Hen No.	Urinary Nitrogen Excretion in Mgms. Total	Uric Acid	Ammonia	Urea	Creatine[1]	Percentage Composition of Urinary Nitrogen Uric Acid	Ammonia	Urea	Creatine[1]	Unknown	Mgms Anthrone[2] Glucose
10/25	1	168.8	85.9	3.6	12.8	21.7	44.8	2.1	7.6	12.8	33	539
10/25	2	226.8	116.9	11.7	20.8	13.7	52.0	5.2	9.2	6.0	27	352
10/25	3	231.6	4.0	13.1	11.7	19.4	44.9	5.7	5.1	8.4	36	374
10/25	4	544.6	158.0	46.9	61.5	23.6	29.0	8.6	11.3	4.3	47	1013
10/25	5	282.8	57.5	72.3	15.8	14.9	20.3	25.6	5.6	5.3	43	319
Average		290.9	82.4	29.5	24.5	18.7	38.2	9.4	7.8	7.4	37	519
10/26	1	243.3	115.6	55.2	17.5	15.8	45.9	22.7	7.2	6.5	16	5041
10/26	2	218.4	103.5	26.9	14.5	18.1	47.4	12.3	6.6	8.6	25	484
10/26	3	236.5	131.6	31.5	13.5	17.2	55.6	13.3	5.7	7.3	18	858
10/26	4	285.2	179.6	6.4	21.0	26.4	63.0	2.2	7.4	9.3	18	330
10/26	5	195.3	61.2	58.7	13.3	19.5	31.3	30.1	6.8	10.0	21	523
Average		235.7	118.3	35.7	15.8	19.4	48.6	16.1	6.5	8.3	20	1147
10/27	1	211.7	59.0	42.8	20.0	20.3	27.9	20.2	9.4	9.6	33	10773
10/27	2	173.6	97.2	18.8	2.4	16.0	56.0	10.8	1.4	9.2	23	275
10/27	3	225.2	112.3	25.3	9.8	21.6	49.9	11.2	4.4	10.0	25	995
10/27	4	323.3	243.2	13.8	3.2	23.9	75.2	4.3	1.0	7.4	12	319
10/27	5	133.7	59.9	6.5	7.3	13.0	44.5	4.9	5.5	9.7	35	2884
Average		213.5	114.3	21.4	8.5	19.0	50.7	10.3	4.3	9.2	26	3049

1 The value for creatine is the combined value of creatine and creatinine
2 Total color compared to glucose standards

(TABLE II CONTINUED)

Date	Hen No.	Urinary Nitrogen Excretion in Mgms. Total	Uric Acid	Ammonia	Urea	Creatine[1]	Percentage Composition of Urinary Nitrogen Uric Acid	Ammonia	Urea	Creatine[1]	Un-known	Mgms. Anthrone[2] Glucose
10/28	1	164.6	82.1	10.8	2.5	16.3	50.0	6.6	1.5	9.9	28	1156
10/28	2	220.8	102.7	25.0	6.5	13.7	46.5	11.3	2.9	6.2	33	242
10/28	3	226.1	97.2	79.7	7.8	19.9	42.9	35.2	3.4	8.8	9	1475
10/28	4	179.1	100.0	14.4	7.2	11.2	55.8	8.0	4.0	6.3	26	286
10/28	5	109.2	41.7	5.0	2.5	11.6	38.2	4.6	2.3	10.6	44	638
Average		180.0	84.7	27.0	5.3	14.5	46.7	13.1	2.8	8.4	28	759
10/29	1	168.3	36.9	9.5	2.6	13.0	21.9	5.6	1.5	7.7	63	440
10/29	2	185.7	25.8	18.6	4.2	11.8	13.9	10.0	2.3	6.3	67	536
10/29	3	190.1	24.9	29.1	7.1	15.6	13.9	15.3	3.7	8.2	60	462
10/29	4	172.9	39.7	9.1	8.6	10.2	22.9	5.3	4.9	5.9	61	591
10/29	5	210.9	9.7	60.4	12.5	20.9	4.5	28.6	5.9	9.9	51	1114
Average		185.6	27.4	25.3	7.0	14.3	13.4	13.0	3.6	7.6	60	629
10/30	1	209.2	70.5	54.1	12.4	19.2	33.7	25.9	5.9	9.2	25	242
10/30	2	232.8	65.5	36.7	7.5	15.3	28.1	15.8	3.2	6.6	46	193
10/30	3	285.6	87.6	44.5	6.3	24.0	30.7	15.6	2.2	8.4	43	165
10/30	4	189.1	45.5	33.3	4.9	11.2	24.1	17.6	2.6	5.9	50	187
10/30	5	318.2	107.4	96.9	15.0	27.9	33.8	30.5	4.7	8.8	23	261
Average		246.8	75.3	53.1	9.2	19.5	30.1	21.1	3.7	7.8	37	210

1 The value for creatine is the combined value of creatine and creatinine.
2 Total color compared to glucose standards

APPENDIX SECTION II

DILUTION TECHNIQUES AND METHODS OF ANALYSIS OF CHICKEN URINE

Dilution Techniques

The urine of the chicken contains various precipitates, the chief one being uric acid. In order to obtain a uniform sample it was found necessary to reduce the size of the granular precipitate to a suspendable size and yet have a minimum of foam. The equipment which accomplished this best was a Servall Omni-mixer (Ivan Sorval, Inc., Norwalk, Conn.). The chilled urine sample was blended at maximum R.P.M. for about 10 minutes, was then drained into a wide mouthed 16 ounce bottle and placed in a freezer to cool and allow the foam to settle. The urine was removed from the freezer before actually freezing and transferred to an eight ounce polyethylene bottle for freezing. Immediately after transfer to the polyethylene bottles, duplicate five or 10 ml. samples were taken for the determination of total nitrogen by the Kjeldahl method; the remainder was frozen and stored at -40° C. Just prior to the determination of the urinary nitrogen components the urines were prethawed in a cold room ($5^{\circ} \pm 3^{\circ}$ C.) for 24 hours and then completely thawed at room temperature in the draft of a window fan for a period of two to three hours. Ten ml. samples were taken for all the determinations immediately after the urines had thawed and the urines were then returned to the freezer. The samples which were not to be analyzed were kept frozen until they were used. In this manner the urines were thawed fewer times.

Folin Ostwald pipettes were found to be superior for the sampling of chicken urine. The variation between duplicate Kjeldahl samples was greatly reduced when serological pipettes were replaced with Folin Ostwald pipettes,

STANDARD TRANSFER PIPETTES WERE FOUND TO BE IMPRACTICAL BECAUSE THE PRECIPITATE CLOGGED THE OPENING.

ALL DILUTIONS WERE MADE FROM 10 ML. SAMPLES TO REDUCE ANY SAMPLING ERROR WHICH MIGHT BE CAUSED BY THE PRECIPITATE PRESENT. DUE TO THE LARGE INITIAL SAMPLES, 1:10 DILUTIONS WERE MADE IN 100 ML. S.T.G.S. PYREX VOLUMETRIC FLASKS.

IN THE DETERMINATION OF AMMONIA, UREA, AND AMINO ACID NITROGEN 0.5 ML. OR 1.0 ML. ALIQUOTS OF UNDILUTED URINE WERE USED. THESE WERE TAKEN WITH 0.5 OR 1.0 ML. FOLIN OSTWALD PIPETTES. IT WAS BELIEVED THAT THE ERROR IN SAMPLING DUE TO THE PRECIPITATE WAS GREATLY REDUCED IN THIS INSTANCE BECAUSE THESE COMPONENTS WERE IN SOLUTION IN THE PRESENCE OF THE BORATE PRESERVATIVE.

URIC ACID WAS DETERMINED ON A 1:1000 DILUTION OF URINE WHICH INVOLVED THE DISSOLVING OF THE URIC ACID. CARBOHYDRATE AND CREATINE WERE RUN ON A 1:100 DILUTION OF URINE.

THE DETERMINATION OF URIC ACID IN CHICKEN URINE

PRINCIPLE

THE METHOD IS BASED ON THE SELECTIVE DESTRUCTION OF URIC ACID BY URICASE. READINGS ARE TAKEN ON A SPECTROPHOTOMETER AT 292 TO 295 M MU BEFORE AND AFTER THE DESTRUCTION OF THE URIC ACID AND THE REDUCTION IN ABSORPTION IS CALCULATED AS URIC ACID.

APPARATUS REQUIRED

A BECHMAN MODEL D.U. SPECTROPHOTOMETER EQUIPPED WITH AN ULTRA-VIOLET ACCESSORY UNIT AND A SEROLOGICAL WATER BATH.

INTRODUCTION

ACCORDING TO STIMSON AND REUTER (1943) THE PURINES HAVE DIFFERENT ABSORPTION PEAKS IN THE NEAR ULTRAVIOLET DUE TO THE HYDROXY GROUPS ATTACHED TO THE CARBONS ADJACENT TO THE NITROGEN ATOMS. SOLUTIONS OF THE THREE PURINES; HYPOXANTHINE, XANTHINE, AND URIC ACID; SHIFT THEIR ABSORPTION MAXIMS TOWARD THE LONGER WAVE LENGTHS AS THE SOLUTION BECOMES MORE ALKALINE. URIC ACID EXHIBITS A MAXIMUM ABSORPTION AT 292 MILLIMICRONS FROM PH 7 TO PH 11. ABOVE PH 7 XANTHINE WILL INTERFERE WITH URIC ACID DETERMINATION BECAUSE THE ABSORBANCE SPECTRUMS OVERLAP AND THE BANDWIDTH OF THE INSTRUMENT ALLOWS SUFFICIENT LIGHT PASSAGE AT SHORTER WAVELENGTH, THE REGION IN WHICH XANTHINE EXHIBITS MORE ABSORPTION.

THE WORK OF PRAETORINS (1948) AND CANLLAKIS AND COHN (1955) WAS USED AS A BASIS FOR THIS METHOD AND ADAPTATIONS WERE MADE TO SUIT THE PECULIAR PROBLEMS FOUND IN ANALYZING CHICKEN URINE.

Procedure

Ten ml. samples of urine were quantitatively transferred to a 1000 ml., pyrex, S.T.G.S., volumetric flask. Fifty mls. of hot lithium borate solution were then added and the flask was gently agitated to dissolve the granular material. (In many urines that have been analyzed there was much precipitate, of undetermined origin, which is insoluble in alkali but soluble in trichloroacetic acid.) After sitting about five minutes the urine is diluted to 1000 ml. with distilled H_2O and thoroughly mixed by inversion. An aliquot of 10 ml. of the diluted urine was transferred to a 100 ml. volumetric flask, made up to volume with distilled water, and an aliquot transferred to a photometer cell. The absorbance was determined at a wave length of 292 m mu using a reagent blank to set the zero absorbance. A standard was prepared by substituting a uric acid solution (5 mg. per ml.) for the urine sample in the original solution.

A uricase treated sample, to determine extraneous absorption was prepared and allowed to incubate while the total absorption was being determined. To five ml. of the final urine dilution in a 20 x 200 mm. culture tube was added two ml. of uricase suspension and three ml. of 0.267 molar borate buffer. The solution was mixed by Rotary agitation and incubated in a water bath at 40° C. for two hours. The absorbance was determined as previously using the same blank for a zero setting. A uricase blank was prepared using five ml. of the original reagent blank instead of the diluted sample of urine; the standards were treated in the same manner. Usually the uricase treated samples absorbed very little more light than the uricase blank and for this reason the clear blank was

USED TO ZERO THE INSTRUMENT. THE URICASE BLANK GAVE A CONSISTENT ABSORPTION OF 0.216 ± 0.001 IN A SERIES OF SEVEN DETERMINATIONS INVOLVING 10 TO 15 SAMPLES.

THE CALCULATIONS OF URIC ACID CONCENTRATION WAS:

$$\frac{U - 2(EU - EB)}{S - 2(ES - EB)} \times (5) \times 1000 = \text{MG. URIC ACID PER ML. OF URINE}$$

U = ABSORBANCE OF UNKNOWN
S = ABSORBANCE OF STANDARD
EU= ABSORBANCE OF ENZYME TREATED UNKNOWN
ES= ABSORBANCE OF ENZYME TREATED STANDARD
EB= ABSORBANCE OF ENZYME BLANK
(S)= CONCENTRATION OF STANDARD IN MCG.

DISCUSSION

SEVERAL METHODS OF DETERMINATION OF URIC ACID WERE TRIED FOR CHICKEN URINE. THE PROPOSED METHOD ALONE GAVE CONSISTENT RESULTS. THE COLORIMETRIC METHODS INVOLVING THE COMMON TUNGSTIC ACID REAGENTS GAVE MORE URIC ACID NITROGEN THAN THERE WAS TOTAL NITROGEN EXCRETED IN THE URINE. THE USE OF CRUDE OX KIDNEY URICASE PREPARATIONS DECREASED THIS APPARENT URIC ACID LEVEL ONLY SLIGHTLY. EVIDENTLY THE CRUDE POWDER OXIDIZED OTHER REDUCING SUBSTANCES PRESENT IN THE URINE. SINCE URICASE FORMS HYDROGEN PEROXIDE WHICH MIGHT ALSO OXIDIZE SOME OF THESE MATERIALS, NO ATTEMPT WAS MADE TO ADAPT THIS METHOD FOR USE WITH ANY OF THE TUNGSTIC ACIDS REAGENTS.

PROBABLY ONE OF THE MOST CRITICAL STEPS INVOLVED WAS THE DILUTION OF THE URINE. FOR THIS REASON LARGE VOLUMES OF SAMPLE AND DILUENT WERE USED. THE URIC ACID IN CHICKEN URINE BEGINS TO PRECIPITATE IN LARGE GRANULES VERY SOON AFTER THE URINE IS VOIDED AND EVEN WITH AN "OMNI-MIXER" THE BEST THAT CAN BE EXPECTED IS A FINE GRANULAR DISPERSION. THE AMOUNT OF LITHIUM BORATE USED IN THIS DILUTION IS SUFFICIENT TO DISSOLVE 100 MG. OF URIC ACID SO THERE IS A GREAT EXCESS FOR THE AMOUNT OF URIC ACID IN 10 ML. OF CHICKEN

URINE FROM A 24-HOUR COLLECTION WHICH HAS ALREADY BEEN ADJUSTED WITH WATER TO A TOTAL VOLUME OF 200 TO 250 ML. ADJUSTMENT OF THE 24-HOUR SAMPLE TO A CONSTANT VOLUME WAS MADE BECAUSE IT IS DIFFICULT TO OBTAIN GRADUATED GLASSWARE IN WHICH THE ORIGINAL UNDILUTED URINE VOLUME MAY BE DETERMINED WITH SUFFICIENT ACCURACY. UNIFORM DILUTION ALSO FACILITATES MACHINE CALCULATIONS.

FOR ROUTINE ANALYTICAL WORK THE MODEL D.U. SPECTROPHOTOMETER IN THIS LABORATORY IS EQUIPPED WITH A TEST TUBE ADAPTER. THE URIC ACID ABSORPTIONS WERE DETERMINED IN 12 X 75 MM. PYREX CULTURE TUBES WHICH HAD PREVIOUSLY BEEN MATCHED AT 292 MILLIMICRONS USING A SOLUTION CONTAINING TWO GRAMS OF URIC ACID PER ML. THIS SOLUTION REQUIRED A SLIT OPENING OF .5 M.M. BUT SLIT WIDTH DID NOT SEEM TO INTERFERE WITH THE SPECIFICITY OF THE METHOD.

THE DETERMINATION OF CREATINE AND CREATININE IN CHICKEN URINE

Reagents Required

Sodium Hydroxide, 0.75 normal

Sodium Hydroxide, 2.0 normal

Note: Both of these may be obtained by diluting a 10 percent solution.

Sodium Picrate Buffer, 11.7 percent pH 2.0 ± 0.05

Apparatus Required

Klett Summerson colorimeter tubes or test tubes graduated at 10 ml. which are matched to within ± 0.001 Optical Density Units.

Beckman Model D.U. spectrophotometer with test tube adapter or a similar instrument.

Preparation of Reagents

Recrystallized picric acid (Benedict, 1929).

100 gms. of picric acid are dried for several hours at 80 - 90° C. The picric acid is dissolved with the aid of heat in 150 ml. of glacial acetic acid, and heating continued until the mixture boils. (The mixture should be heated in an Erlenmeyer flask upon an electric hot plate.) Pour the hot solution upon a fluted filter contained in a dry funnel which has been previously heated, and collect the filtrate in a dry beaker. Cover the beaker with a watchglass and allow to stand for some hours, or overnight at room temperature (not in a refrigerator). At the end of this time if picric acid has not crystallized out, stir the mixture vigorously, or better, seed with a minute crystal of pure picric acid. Crystallization

WILL BEGIN AT ONCE AND IS COMPLETE WITHIN TWO HOURS OR LESS. AT THE END OF TWO HOURS, FILTER WITH SUCTION ON A HARDENED FILTER PAPER AND WASH WITH ABOUT 35 ML. OF COLD GLACIAL ACETIC ACID. SUCK AS FREE OF ACETIC ACID AS POSSIBLE AND DRY AT ABOUT 80 - 90° C. WITH OCCASIONAL STIRRING, UNTIL THERE IS NO ODOR OF ACETIC ACID. ALLOW AN AIR VENT IN THE OVEN.

SATURATED PICRIC ACID

TO 11.7 GMS. PICRIC ACID IN A ONE LITER VOLUMETRIC FLASK ADD BOILING HOT DISTILLED WATER TO THE NECK OF THE FLASK. SHAKE UNTIL DISSOLVED. SET ASIDE TO COOL AND BRING TO VOLUME (PETERS, 1942).

THE PICRIC ACID MUST PASS THE FOLLOWING TEST (FOLIN AND DOISY, 1917). TO 10 ML. OF THE SATURATED PICRIC ACID SOLUTION (SEE ABOVE FOR PREPARATION), ADD 0.5 ML. OF 10 PERCENT SODIUM HYDROXIDE AND LET THE MIXTURE STAND 15 MINUTES. THE COLOR OF THE ALKALINE PICRATE SOLUTION THUS MADE MUST NOT BE MORE THAN TWICE AS DEEP AS THE COLOR OF THE SATURATED PICRIC ACID SOLUTION. IF A DEEP COLOR DOES FORM IN ALKALINE SOLUTION, IMPURITIES ARE PRESENT WHICH FORM SUFFICIENT COLOR TO INTERFERE WITH CREATININE DETERMINATIONS (MCCRUDDEN AND SARGENT, 1916).

SODIUM PICRATE BUFFER

ONE LITER OF THE 1.17 PERCENT PICRIC ACID SOLUTION IS ADJUSTED TO pH 2.0 $\pm$ 0.05 BY THE ADDITION OF APPROXIMATELY 20 ML. OF 2 N SODIUM HYDROXIDE. IT IS ADVISABLE TO CHECK THE pH OF THE SOLUTION AFTER SEVERAL HOURS BECAUSE THE FRESHLY PREPARED SOLUTION TENDS TO DRIFT IN pH.

SODIUM HYDROXIDE SOLUTION

100 GMS. OF C.P. SODIUM HYDROXIDE PELLETS ARE DISSOLVED IN ONE LITER OF WATER AND STORED IN A PYREX BOTTLE. TO PREPARE 2 N NaOH DILUTE 40 ML. OF THE

10 PERCENT NaOH TO 50 ML. WITH DISTILLED WATER. TO PREPARE 0.75 N SOLUTION, DILUTE 300 ML. OF THE 10 PERCENT NaOH TO ONE LITER.

CREATININE STANDARD

ALTHOUGH CREATININE MAY BE OBTAINED IN A HIGHLY PURE FORM, THE PUREST STANDARDS MAY BE PREPARED FROM CREATININE ZINC CHLORIDE.

WEIGH OUT 2.1660 GMS. OF CREATININE ZINC CHLORIDE, PLACE IN A 500 ML. VOLUMETRIC FLASK AND MAKE UP TO VOLUME WITH 0.1 N HCL. THIS STOCK STANDARD SHOULD BE KEPT IN REFRIGERATOR; IT CONTAINS ONE MG. OF CREATININE NITROGEN OR 3.201 MG. OF CREATININE PER ML. AND IS STABLE FOR MONTHS IN THE REFRIGERATOR.

WORKING STANDARDS

THE WORKING STANDARDS ARE PREPARED BY DILUTING THE STOCK STANDARD AT THE TIME OF USE. THE METHOD IS EXTREMELY SENSITIVE WITH A RANGE OF ONE TO 20 GAMMAS OF CREATININE PER ML. OF DILUTED STANDARD. ALL SAMPLES SHOULD BE DILUTED IN SUCH A MANNER AS TO LIE IN THIS RANGE. WORKING STANDARDS ARE NOT STABLE.

PROCEDURE

CREATININE

USING A FOLIN-OSTWALD PIPETTE TRANSFER 10 ML. OF FRESHLY BLENDED URINE TO A 15 ML. CENTRIFUGE TUBE. ADD 1 ML. OF 30 PERCENT TRICHLOROACETIC ACID AND CENTRIFUGE FOR 10 MINUTES AT 1500 R.P.M. POUR THE SUPERNATANT INTO ONE LITER VOLUMETRIC FLASK, FILL THE FLASK TO VOLUME WITH WATER AND MIX BY INVERSION.

PIPETTE 5 ML. OF THE DILUTED URINE INTO TEST TUBES GRADUATED AT 10 ML. (IN THIS LABORATORY KLETT SUMMERSON COLORIMETER TUBES WERE USED.) TO THE SAMPLE IN THE TEST TUBE ADD TWO ML. OF PICRATE BUFFER FOLLOWED BY TWO ML.

Of 0.75 sodium hydroxide. A syringe pipette may be used to speed up the determination. Dilute to 10 ml. immediately, mix well, and allow to stand 30 minutes at room temperature for the color to develop. The optical density is then determined on the Beckman Model D.U. spectrophotometer at a wave length of 495 millimicrons and a slit opening of 0.03 m.m.

The blank is prepared by substituting water for the diluted urine. A standard of convenient dilution should be prepared and run through the procedure with the samples. In this laboratory the readings were made in the tubes using a Beckman Model D.U. spectrophotometer equipped with a test tube adapter. This was found to be a highly satisfactory arrangement allowing about 30 or 40 determinations per hour with little or no sacrifice of accuracy.

Creatine

For the creatine determination the sample and picrate buffer are added to the graduated tubes as for the creatinine determination, then the tubes are either autoclaved for 45 minutes at 20 lbs. pressure or are placed in a vigorously boiling water bath for two hours. The tubes are then allowed to cool to room temperature and the volume is adjusted to about 5 ml. with water. After the volume has been adjusted, add 2 ml. of 0.75 N sodium hydroxide and then dilute to 10 ml. with water. Allow to stand 30 minutes and determine the absorbance as with creatinine.

Discussion

It was found in this laboratory that the color produced by creatinine followed the Beer-Lambert Law in that portion of the spectrum between 480

AND 510 MILLIMICRONS WHEN THE CONCENTRATIONS OF CREATININE RANGES FROM ONE TO 20 MICROGRAMS PER ML. OF SAMPLE.

PROBABLY THE FIRST PREREQUISITE FOR SUCCESS WITH THIS METHOD IS THE PROPER PURIFICATION OF THE PICRIC ACID. THE 10 PERCENT SODIUM HYDROXIDE WAS USED BECAUSE IT WAS DESIRABLE TO USE THE SAME SODIUM HYDROXIDE FOR ALL THE DETERMINATIONS INVOLVED IN THE EXPERIMENT. ONE PARTICULAR PROBLEM IN THE CREATINE DETERMINATION WAS THE PRESENCE OF "HOT SPOTS" IN THE GAS HEATED WATER BATH. FOR THIS REASON A COVERED, ELECTRICALLY HEATED BATH IS RECOMMENDED IF AN AUTOCLAVE IS NOT AVAILABLE.

THE DETERMINATION OF AMMONIA AND UREA NITROGEN IN CHICKEN URINE[1]

Procedure for Ammonia

Aeration Method (Van Slyke and Cullen, Modified): Principle.

The urine is treated with a saturated potassium carbonate solution, and the liberated ammonia is transferred by aeration into an acid receiving solution, where it is then determined by titration. In the original procedure, the ammonia is aerated into 0.02 N acid which is then back-titrated with 0.02 N alkali. In the modification described here, 0.5 percent (v/v) sulfuric acid is used to receive the ammonia and is then directly Nesslerized.

Apparatus Required

Beckman Model D.U. spectrophotometer or other photoelectric instrument.

Aeration apparatus - The apparatus used for the work reported in this dissertation was made in such a manner that 24 determinations could be made simultaneously. The 24 sets of aeration tubes were hooked in parallel to two manifolds. (See Figures II-1 and II-2.) Each aeration train had its own clamp for adjusting air flow. The receiving tubes were aerated through gas diffusing tubes (C.G. No. 39533). The diffusing tubes cause smaller bubbles to be formed which should allow a better absorption of the ammonia by the acid.

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[1]As adapted from Hawk et al. (1954)

Reagents Required

50 percent potassium carbonate

0.5 percent (v/v) sulphuric acid

Nessler's reagent (Koch and McMeekin)

Procedure

Measure 0.5 to 1.0 ml. of undiluted urine into one of the two large test tubes used in an aeration train and connect this tube for aeration, as shown in the illustrations, with a second tube containing 15 ml. of the 0.5 percent sulfuric acid. At this time a blank is prepared by substituting water for the aliquot of urine. Add a drop of caprylic alcohol to each tube to minimize foaming. When ready, remove the stopper of the tube containing the urine and add 5 ml. of 50 percent potassium carbonate solution. Replace the stopper tightly and start the air current (pressure or suction; the incoming air must be washed by preliminary passage through a wash-bottle containing dilut (1:10) sulfuric acid to remove any ammonia present). The air current should be run slowly for the first two minutes, and then increased to aerate as fast as the apparatus will stand. Aeration is continued until all the ammonia has been driven over; this may take from five to 30 minutes, depending upon the apparatus, and the time required should be established by trial.

When aeration is complete, remove the tube containing the sulfuric acid, rinsing down the inlet tube in the process, and transfer the contents to a 50 ml. volumetric flask. Add 25 ml. of water followed by .5 ml. of Nessler's reagent. Dilute the flask to volume with water and mix by inversion. Determine the absorbance in a photometer at a wave length of 480 millimicrons. Set the photometer to zero absorbance with the reagent blank. Standard

SOLUTIONS CONTAINING 0.2 TO 0.5 MG. OF AMMONIA NITROGEN ARE RUN AT THE SAME TIME AS THE URINE SAMPLES.

PROCEDURE FOR UREA

PRINCIPLE

THE URINE SAMPLE IS TREATED WITH UREASE, AND THE AMMONIA FORMED IS AERATED INTO 0.5 PERCENT (V/V) SULFURIC ACID, WHICH IS THEN DIRECTLY NESSLERIZED.

APPARATUS REQUIRED

BECKMAN MODEL D.U. SPECTROPHOTOMETER OR OTHER PHOTOELECTRIC INSTRUMENT.

AERATION APPARATUS DESCRIBED FOR THE AMMONIA DETERMINATION.

REAGENTS REQUIRED

50 PERCENT POTASSIUM CARBONATE

0.5 PERCENT (V/V) SULFURIC ACID

NESSLER'S REAGENT (KOCH AND MCMEEKIN)

PHOSPHATE BUFFER SOLUTION - SIX GMS. OF ACID POTASSIUM PHOSPHATE (KH_2PO_4) AND 5 GMS OF DISODIUM PHOSPHATE ($Na_2HPO_4 . 12H_2O$) ARE DISSOLVED IN WATER AND MADE UP TO ONE LITER.

UREASE SUSPENSION - THE WORKING SUSPENSION WAS MADE BY DILUTING 10 ML. OF A CONCENTRATED UREASE PREPARATION TO 50 ML. WITH PHOSPHATE BUFFER. THE CONCENTRATED SUSPENSION WAS MADE AS FOLLOWS:

GLYCEROL EXTRACT (KOCH) - PLACE 37.5 GM. OF PERMUTIT IN A LITER FLASK. ADD 500 ML. OF TWO PERCENT ACETIC ACID. SHAKE WELL. POUR OFF THE LIQUID. WASH THE PERMUTIT IN THE FLASK WITH TWO 300 ML. PORTIONS OF DISTILLED WATER, DECANTING OFF THE LIQUID EACH TIME. ADD 75 GM. OF JACKBEAN MEAL TO THE PERMUTIT IN THE FLASK. ADD 125 ML. OF 0.001 N SULFURIC ACID AND SHAKE GENTLY AT TEN MINUTE INTERVALS OVER

FIGURE II-1

THE AERATION APPARATUS FOR THE DETERMINATION OF AMMONIA

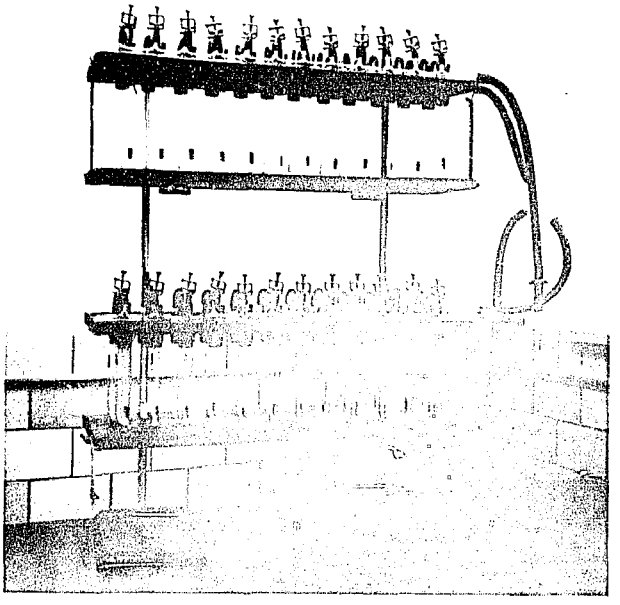
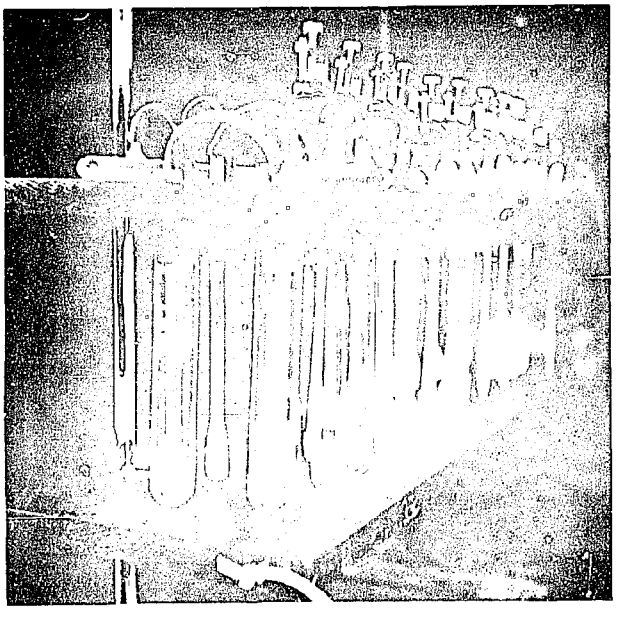


FIGURE II-2

CLOSE-UP VIEW OF AERATION APPARATUS SHOWING CONSTRUCTION DETAILS



THE COURSE OF AN HOUR. ADD 375 ML. OF REAGENT GRADE GLYCERIN. MIX WELL. FILTER THROUGH A FLUTED FILTER PAPER INTO AN AMBER BOTTLE. THIS PREPARATION IS STABLE FOR A YEAR AT ROOM TEMPERATURE AND WILL KEEP INDEFINITELY IN THE REFRIGERATOR.

THE PREPARATION USED IN THIS LABORATORY WAS MADE BY SUBSTITUTING 25 GMS. OF A COMMERCIAL UREASE POWDER (SIGMA CHEMICAL CO., ST. LOUIS, MO.) FOR THE JACKBEAN MEAL. THE RESULTING PRODUCT HAD A HIGH ACTIVITY AND GAVE A VERY LOW BLANK.

PROCEDURE

MEASURE 0.1 TO 1.0 ML. OF URINE INTO A LARGE TEST TUBE SUITABLE FOR AERATION, ADD 1 DROP OF CAPRYLIC ALCOHOL (TO PREVENT FROTHING), AND 1 ML. OF DILUTE ENZYME SOLUTION. CLOSE THE TUBE WITH A RUBBER STOPPER AND LET THE TUBE STAND 15 MINUTES FOR THE ENZYME TO ACT. A BLANK IS PREPARED WITH EACH SET OF URINE SAMPLES. MEASURE INTO A SECOND SIMILAR TUBE 15 ML. OF 0.5 PERCENT H_2SO_4. ADD 1 DROP OF CAPRYLIC ALCOHOL AND CONNECT THE TUBE FOR AERATION WITH WASHED AIR BY EITHER PRESSURE OR SUCTION. AT THE END OF 15 MINUTES OPEN THE TUBE CONTAINING THE SAMPLE AND INTRODUCE 5 ML. OF 50 PERCENT POTASSIUM CARBONATE. PLACE THE TUBE ON THE AERATION TRAIN AT ONCE AND AERATE UNTIL ALL THE AMMONIA HAS BEEN CARRIED OVER INTO THE ACID IN THE RECEIVER. THE TIME NEEDED FOR THE AERATION VARIES FOR DIFFERENT PUMPS FROM 5 TO 30 MINUTES, AND SHOULD BE DETERMINED BY TRIAL FOR THE PARTICULAR APPARATUS USED. AT THE END OF THE TIME NEEDED FOR THE AERATION, THE PUMP IS DISCONNECTED (CARE BEING TAKEN TO AVOID BACK SUCTION) AND THE ACID IN THE RECEIVER IS TRANSFERRED TO A 50 ML. VOLUMETRIC FLASK. IT IS THEN TREATED IN THE SAME MANNER AS WAS DONE IN THE AMMONIA DETERMINATION.

Discussion

The method works very well with chicken urine. Anyone using this method should adjust the volume of the aliquot of urine in order to prevent precipitation in the Nesslerizing procedure, which happens when too much ammonia is present. The blanks were very low when the air flow through the trains was properly adjusted and when the bubbles were washed carefully after each determination.

Pressure, rather than vacuum was used for aeration because the pressure cleaned all liquid out of the bubbles when the tubes were removed. The receiving tubes were always removed first.

This method was chosen because there is less doubt that the final result is due to the presence of urea and ammonia.

THE DETERMINATION OF CARBOHYDRATE IN CHICKEN URINE[2]

PRINCIPLE

THE SAMPLE IS TREATED WITH CONCENTRATED SULFURIC ACID AND A SOLUTION OF ANTHRONE IN ETHYL ACETATE. THE CARBOHYDRATES DEVELOP A CHARACTERISTIC GREEN COLOR AND CARBOHYDRATE CONCENTRATION IS ESTIMATED PHOTOMETRICALLY.

APPARATUS REQUIRED

A BECKMAN MODEL D.U. SPECTROPHOTOMETER OR A COMPARABLE PHOTOELECTRIC INSTRUMENT.

REAGENT REQUIRED

CONCENTRATED SULFURIC ACID.

2 PERCENT SOLUTION OF ANTHRONE IN REAGENT GRADE OR C.P. ETHYL ACETATE.

PROCEDURE

TO 2 ML. OF THE CARBOHYDRATE SOLUTION (CONTAINING AN AMOUNT OF CARBOHYDRATE THAT WILL GIVE A COLOR INTENSITY IN THE RANGE GIVEN BY 0 AND 80 MICROGRAMS OF GLUCOSE) IN A 19 X 150 MM. TEST TUBE IS ADDED 0.5 ML. OF A SOLUTION OF 2 PERCENT ANTHRONE (RECRYSTALLIZED FROM BENZENE AND LIGHT PETROLEUM ETHER) IN REAGENT GRADE OR C.P. ETHYL ACETATE. THEN 5 ML. OF CONCENTRATED SULFURIC ACID ARE CAREFULLY LAYERED INTO THE TUBE. THE TUBE IS GENTLY SWIRLED UNTIL THE ETHYL ACETATE HAS HYDROLYZED, AS INDICATED BY THE FLOC OF ANTHRONE WHICH APPEARS. MORE RAPID SWIRLING THEN THOROUGHLY MIXES THE CONTENTS OF THE TUBE AND DISSOLVES THE ANTHRONE. THE DEVELOPED COLOR MAY BE READ AFTER 10 MINUTES AT 620 MILLIMICRONS IN A SPECTROPHOTOMETER AGAINST DISTILLED WATER AND CORRECTED FOR THE ABSORPTION OF A BLANK CONTAINING

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[2] ADAPTED FROM THE METHOD OF LOEWUS (1952)

ONLY THE REAGENTS AND WATER.

A PLOT OF THE OPTICAL DENSITY AGAINST THE WEIGHT OF GLUCOSE IN THE SAMPLE GIVES A STRAIGHT-LINE RELATIONSHIP FROM 0 - 80 MICROGRAMS. THE ACETIC ACID AND ETHYL ALCOHOL PRODUCED BY THE HYDROLYSIS OF THE ETHYL ACETATE DO NOT INTERFERE WITH THE REACTION IN ANY WAY. THE ETHYL ACETATE SOLUTION OF ANTHRONE WILL KEEP SEVERAL WEEKS WHEN STORED IN AN AMBER GLASS-STOPPERED BOTTLE. THUS, THE TROUBLESOME DEVELOPMENT OF COLOR IN THE SULFURIC ACID-ANTHRONE REAGENT EMPLOYED IN EARLIER PROCEDURES IS AVOIDED.

Discussion

THE PROPER DILUTION OF URINE WAS FOUND TO BE 1:100 FOR THIS DETERMINATION AND ALIQUOTS OF THE SAME DILUTED SAMPLE WAS USED FOR THE CARBOHYDRATE DETERMINATION AND THE CREATINE DETERMINATION. THE REACTION WAS CARRIED OUT IN BLOOD SUGAR TUBES AND THE COLORED MATERIAL WAS THEN TRANSFERRED TO KLETT SUMERSON COLORIMETER TUBES FOR THE DETERMINATION OF THE ABSORBANCY.

THE VALUES WERE EXPRESSED AS EQUIVALENTS OF MILLIGRAMS OF GLUCOSE STANDARDS. THE METHOD GAVE VERY UNIFORM RESULTS. THE COEFFICIENT OF VARIATION OF 30 SETS OF DUPLICATE DETERMINATIONS ON URINE SAMPLES WAS 0.5 PERCENT.

THE DETERMINATION OF AMINO ACID NITROGEN IN CHICKEN URINE

Principle

THE URINES ARE DESALTED ON AN ION-EXCHANGE COLUMN TO REMOVE AMMONIA, UREA, AND OTHER COMPOUNDS, WHICH REACT WITH NINHYDRIN, AND THE AMINO ACID NITROGEN IS DETERMINED ON THE ELUATE.

Apparatus Required

BECKMAN MODEL D.U. SPECTROPHOTOMETER OR A COMPARABLE INSTRUMENT.

STEAM BATH OR FORCED DRAFT OVEN.

CONSTANT BOILING, ELECTRIC WATER BATH.

Reagents Required

2 N HYDROCHLORIC ACID

2 N SODIUM HYDROXIDE

4 N ACETIC ACID

0.2 PERCENT NINHYDRIN IN 95 PERCENT ETHANOL

0.3 PERCENT DISODIUM EDTA BUFFER pH

Procedure

THE UREA, AMMONIA, AND VARIOUS CATIONS ARE REMOVED BY THE METHOD OF AWAPARA AND SATO (1956). THE URINES ARE DECOLORIZED BY ADDING 0.5 OF CHARCOAL TO 5 ML. OF URINE; TWO TO FIVE DROPS OF CHLOROFORM ARE THEN ADDED TO THE MIXTURE. THE CHLOROFORM IS NECESSARY TO PREVENT ABSORPTION OF TYROSINE, PHENYLALANINE AND TYPTOPHAN BY THE CHARCOAL. AFTER MIXING WELL THE MIXTURE IS WARMED ON A STEAM BATH FOR FIVE MINUTES AND THEN FILTERED. THE FILTRATE IS USUALLY A CLEAR FLUID WITH A YELLOW CAST.

FIVE ML. OF THE CHARCOAL TREATED URINE ARE CONCENTRATED IN A STEAM BATH OR FORCED DRAFT OVEN SET 60° C. TO ABOUT ONE ML. (THE VOL. NEED NOT BE EXACT). OFTEN SOME INSOLUBLE PARTICLES WILL APPEAR UPON CONCENTRATION. THE CONCENTRATED URINE IS NOW READY TO BE DE-SALTED. DOWEX -2 RESIN (200 - 400 MESH) IS PURIFIED BY SUSPENDING THE RESIN IN 2N HCl AND HEATING THE SUSPENSION IN A STEAM BATH FOR TWO HOURS. THE RESIN IS FILTERED THROUGH A BUCHNER FUNNEL AND WASHED UNTIL NEUTRAL. A LARGE QUANTITY CAN BE PREPARED AT ONE TIME AND STORED IN THE CHLORIDE FORM. PRIOR TO USE, A QUANTITY OF THE RESIN IS CONVERTED TO THE HYDROXIDE FORM. THIS IS EASILY DONE BY SUSPENDING THE RESIN IN WARM 2N NaOH FOR ABOUT 30 MINUTES, THE RESIN IS FILTERED AND WASHED THOROUGHLY WITH COLD, BOILED DISTILLED WATER UNTIL THE WASHING IS NEUTRAL. THE COLUMNS ARE PREPARED BY SUSPENDING THE RESINS (ONE PART RESIN TO THREE PARTS WATER) IN BOILED DISTILLED WATER AND POURING A CERTAIN AMOUNT OF THE SUSPENSION IN THE COLUMN (13 X 1CM.). RESIN IS ADDED OR TAKEN FROM THE COLUMN UNTIL THE RESIN BED IS 3 CM. LONG. THE RESIN IS WASHED ONCE WITH WATER. A SMALL PAD OF COTTON WOOL IS PLACED ON TOP OF THE RESIN BED. THE CONCENTRATED URINE IS TRANSFERRED WITH A LONG TIP MEDICINE DROPPER TO THE TOP OF THE RESIN BED. THE CONTAINER IS RINSED WITH 0.5 ML. OF WATER AND THE WASHINGS TRANSFERRED TO THE COLUMN. WHEN ALL THE URINE HAS ENTERED THE RESIN BED, WASHING IS STARTED. THE WASHING, WITH BOILED, COLD DISTILLED WATER, IS CONTINUED UNTIL THE FILTRATE, WHICH IS AT FIRST STRONGLY BASIC, BECOMES NEUTRAL. THE ELUTION OF AMINO ACIDS IS ACCOMPLISHED BY THE USE OF 4 N ACETIC ACID. ACID IS ADDED TO THE COLUMN UNTIL 10 ML. OF ELUATE HAVE BEEN COLLECTED. THE ELUATE IS EVAPORATED TO DRYNESS IN THE STEAM BATH OR FORCED DRAFT OVEN (60° C.) AND THE RESIDUE

TAKEN UP IN 5 ML. WATER. THE pH OF THIS SOLUTION SHOULD BE ABOUT FOUR TO FIVE. IF IT IS MORE ACID, THE EVAPORATION SHOULD BE REPEATED AND THE RESIDUE TAKEN UP IN 5 ML. WATER.

THE AMINO ACID NITROGEN CONCENTRATION IS DETERMINED BY THE METHOD OF MEYER (1957). TO 0.1 ML. OF THE ELUATE IN A PLASTIC CAPPED CULTURE TUBE IS ADDED 0.2 ML. OF 0.3 PERCENT EDTA BUFFER AND 4 ML. OF 0.2 PERCENT NINHYDRIN IN ETHANOL. THE TUBES ARE TIGHTLY CAPPED AND PLACED IN A VIGOROUSLY BOILING WATER BATH FOR 25 MINUTES. THE TUBES ARE THEN REMOVED AND ALLOWED TO COOL TO ROOM TEMPERATURE. AFTER COOLING THE COLORED SOLUTIONS ARE TRANSFERRED TO A PHOTOMETER TUBE AND THE ABSORBANCE DETERMINED AT 578 MILLIMICRONS USING A REAGENT BLANK TO ZERO THE INSTRUMENT.

THE ABSORBANCY IS COMPARED TO THAT OF A STANDARD CONTAINING FROM 0.6 TO 60 MCGS. OF AMINO ACID NITROGEN PER ML. MIXED STANDARDS CONTAINING GLYCINE, ALANINE, CYSTINE, METHIONINE, LYSINE AND ARGININE WERE MADE UP ON AN EQUAL NITROGEN BASIS AND USED IN THIS LABORATORY.

Discussion

THE DE-SALTING TECHNIQUE WAS TIME CONSUMING AND THE NINHYDRIN REACTION WAS SOMEWHAT DIFFICULT TO MASTER. THE DE-SALTING WAS NECESSARY BECAUSE THE AMMONIA AND UREA IN THE CHICKEN URINES GAVE A GREAT DEAL OF COLOR WITH NINHYDRIN. THE NINHYDRIN TECHNIQUE DID NOT GIVE CONSISTENT RESULTS UNTIL THE EDTA HAD BEEN RECHRYSTALLIZED FROM WATER AND THE 95 PERCENT ETHANOL HAD BEEN REDISTILLED OVER POTASSIUM HYDROXIDE.

THE AMINO ACID NITROGEN CONCENTRATION IN CHICKEN URINE WAS FOUND TO BE VERY SMALL AND THE METHOD IS VERY SENSITIVE. THIS SEEMED TO BE A VERY USEFUL COMBINATION.

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AUTOBIOGRAPHY

I was born in Chicago, Illinois on July 2, 1927, the first son of O.G. Richardson and Jennie Owen Richardson, formerly of Magnolia, Arkansas. When I was two years old we moved back to a farm just out of Magnolia where my family still resides.

I started to grammar school in the old Southwest Academy at Magnolia in 1934. I graduated from Magnolia High School in June, 1945. I promptly joined the Navy and stayed in the Navy untill September 6, 1951. While I was in the Navy I married the former Virginia Lewis of Little Rock. Our first child, a girl named Janet Ruth, was born on March 31, 1951. Four years later on March 31, 1955 we welcomed our second child and first son whom we named Charles Edward Richardson II.

On September 11, 1951 I entered Southern State College, Magnolia, Arkansas. I attended that institution till February, 1953 when I transferred to Louisiana State University. I completed my B.S. in General Agriculture in the spring of 1954.

I entered the Graduate school of the Louisiana State University to work toward a Master of Science Degree in Poultry Husbandry in the fall of 1954. I received my Master of Science Degree in June 1956, and immediately began a program of work designed to meet the requirements for a Doctor of Philosophy Degree at Louisiana State University.

EXAMINATION AND THESIS REPORT

Candidate: CHARLES E. RICHARDSON

Major Field: POULTRY NUTRITION

Title of Thesis: THE EFFECT OF DIETARY PROTEIN AND ENERGY LEVEL UPON THE NITROGEN COMPONENTS IN THE URINE OF THE DOMESTIC HEN

Approved:

A B Watts
Major Professor and Chairman

Richard J. Russell
Dean of the Graduate School

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Date of Examination:

JANUARY 13, 1959